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Immunological Studies on the Genus Spirometra (Cestoda: Pseudophyllidea).

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IMMUNOLOGICAL STUDIES ON THE GENUS SPIROMETRA (CESTODA: PSEUDOPHYLLIDEA)

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES
(continued)

LIST OF FIGURES

LIST OF PLATES

ABSTRACT

Total serum proteins were determined, electrophoresis and double gel diffusion were performed on the sera from animals infected with the plerocercoids or passively immunized with larval antigen from Spirometra mansoni, S. urichi and S. mansonoides "A" and "B".

Serum samples from mice were taken at three, 12 and 24 hours post infection and at four day intervals thereafter for a period of 12 weeks. Sera from these groups analyzed by electrophoresis exhibited decreased serum albumin concentrations and generally increases in the alpha, beta and gamma globulins.

Rabbits passively immunized against the plerocercoids of these species exhibited little change in the total proteins after immunization. Changes in the protein fractions were expressed as slight decreases in albumin and a corresponding increase in the gamma globulins.

Precipitin bands were demonstrable by double gel diffusion in sera from all groups of mice infected with the plerocercoids of the three species of worms.

Sera from rabbits passively immunized against the three species formed a maximum of three precipitin bands in agar gel six weeks post immunization.

Intradermal tests in mice passively immunized against Spirometra mansoni and S. urichi gave widely variable results. Reactions obtained with injection of larval antigens indicate the presence of delayed hypersensitivity.

INTRODUCTION

Faust, et al. (1929) divided the genus Diphyllobothrium Cobbold, 1858, into two subgenera, Diphyllobothrium and Spirometra, based on uterine configuration and the shape of the eggs. Diphyllobothrium was to include those species with the outer uterine coils forming a rosette and eggs with rounded ends. The subgenus Spirometra was to include those species with the outer uterine coils spiralled and eggs with pointed ends. Mueller (1937) elevated Spirometra to generic rank and further suggested the establishment of two other genera, subsequently named Dibothriocephalus and Cordicephalus by Wardle, et al. (1947). The cirrus, vagina and uterus open separately in Spirometra, whereas, the other two genera have common cirro-vaginal atria and separate pores. As further evidence for separation, Mueller (1937) noted that the coracidia of Diphyllobothrium utilize the copepod Diaptomus as an intermediate host, while Spirometra coracidia are infective to species of Cyclops.

Other biological characteristics differ in respect to these genera. Plerocercoids of Spirometra are infective to a wide range of vertebrate hosts including amphibians, reptiles and mammals, but never in fish, whereas, the plerocercoids of the diphyllobothrids utilize fish.

Wardle and McLeod (1952) list 16 species for the genus Spirometra, some of which are of questionable taxonomic status. Some of these occur in widely separated geographic localities, while others are found sympatrically. It has been observed by this author in life

cycle and morphological studies with Spirometra, that in the same strobila, it may be possible to find different proglottids that could be identified with several species of this genus. It may be further noted that in lineage studies with a "species" of Spirometra, utilizing the same laboratory cat (Felis catus) as the host, strobila were obtained that have different morphological appearances. This variability complicates species diagnosis.

The pseudophyllidean cestode, Spirometra, utilizes a variety of animal species for its intermediate and definitive hosts. Free-swimming coracidia hatch from eggs and are ingested by copepods of the genus Cyclops. The ingested coracidium sheds the ciliated embryophore and burrows through the gut into the haemocoel, where it develops into a procercoid. When the procercoids are ingested by suitable amphibians, reptiles or mammals they develop into plerocercoids in the fascia of the skin and muscle. Should plerocercoids or infected intermediate hosts be consumed by animals not suitable as a definitive host, the plerocercoids will migrate through the gut and re-establish in the somatic tissues. This may be repeated time and again with apparent impunity until the suitable definitive host is involved. The definitive hosts are members of the family Felidae. When plerocercoids are consumed by the definitive host, the scolex attaches, the posterior portion of the body is shed and development into an adult worm begins. Adult worm infections have also been noted in dogs (Canis familiaris) and raccoons (Procyon lotor).

The host-parasite relationship of spirometrid plerocercoids in experimental animals is unique. Mueller (1963, 1965a, 1965b) noted that mice and hamsters infected with the plerocercoids of S.

mansonoides consistently exhibit weight gains not shown in uninfected animals.

Little study has been devoted to the detection of immune responses in plerocercoid infected animals. Mueller (1961) observed that immune precipitates form around the scolices and bodies of plerocercoids when placed in serum from chronically infected mice. He stated that the first month of infection is the period of antibody production, however, no explanation or evidence is given for the detection of the antibodies. Sadun, et al. (1965) examined the biochemical changes that occur in mice infected with plerocercoids of S. mansonoides. They were unable to detect any immune responses utilizing serum electrophoresis. Mueller (1969) concluded that mice acquired immunity to challenges with procercoids as a result of an existing infection with plerocercoids.

In my laboratory more than an occasional host reaction to the presence of plerocercoids has been observed. These reactions ranged from very subtle ones to regions of necrosis.

The purpose of this study was to examine the possible immune responses in experimental animals actively and passively immunized with the plerocercoids of three species of Spirometra. It was also the hope that analysis of immune responses might be used as an aid to delineate the species. The species used in this study were S. mansonoides "A" and "B" from Louisiana, S. urichi from Florida, and a S. mansoni-like form from Panama, hereafter referred to as S. mansoni. Plerocercoids for all species except S. mansonoides "B" were cultured from eggs obtained from laboratory cats infected with the respective species. Spirometra mansonoides "B" plerocercoids were obtained from

snakes (Lampropeltis getulus holbrooki, Natrix sipedon confluens and N. erythrogaster flavigastor) in West Baton Rouge Parish. The plerocercoids from snakes were chosen because of the virulence they exhibited when transferred to mice.

The parasitologist has long been aware that animals respond in a variety of ways when infected with protozoan and metazoan parasites. The responses range from mortality of the infected host, to states of being refractory or absolutely immune to reinfection with the same organism. Some infections elicite little or no response. Taliaferro (1929) notes that parasites act as any other infectious agent residing in the body of animals, eliciting cellular and humoral responses.

Studies of host-parasite relationships have been undertaken in the hope of better understanding the mechanisms engendered by the host in repelling or allowing survival of parasitic infections.

There are available a variety of tests to measure immune responses. Among the most widely used are studies of acquired immunity, the intradermal injection of antigens in sensitized animals, the complement-fixation test, precipitin test, gel diffusion techniques, immunoelectrophoresis and the quantitative determination of serum protein component changes. So that the data of this study can be better understood, a review of earlier works is in order.

Acquired Immunity

Immunological responses are manifested in a number of ways, among these is acquired immunity. Following initial exposure to a parasitic infection or by injection of nonliving materials derived from the parasite or immune serum, animals may become refractory to subsequent challenges. Acquired resistance may be of an active or passive nature. The former, as a result of an active infection, is generally slow to develop but results in a lasting protection. The latter, resulting from injections of worm material or immune serum affords less protection. However, if immune serum is used it is more quickly effective but is transient. Acquired resistance may be demonstrated in a host by a reduction in the number of established parasites, interference with the normal growth and reproduction or termination of the infection before it becomes patent.

Nematoda. Prior infections with large or graded series of Ancylostoma canium larvae have been shown to impart an immunity in dogs when subsequently exposed to challenge doses which proved fatal to previously unexposed animals (Sandground, 1927; Sarles, 1929; McCoy, 1931a; Foster, 1935; Otto and Kerr, 1939; Otto, 1941).

Stoll (1929, 1932) studied the resistance acquired by animals allowed to become increasingly infected with nematodes under natural conditions. Rabbits and lambs became heavily infected as reflected by increasing egg counts. This was followed in turn by a drop in egg counts and an explusion of the worms. These animals were protected from any significant amount of reinfection. Mayhew's (1940, 1941) studies on bovine gastro-intestinal nematodes indicated that immunity was conferred by previous infections as reflected by reduced egg

counts following reinfection.

Initial infections with Nippostrongylus muris in rats enhanced resistance to subsequent challenges (Chandler, 1932, 1935; Graham, 1934). Graham found that the degree of resistance was related to the size of the initial infecting dose. Whereas Chandler noted that progressive increase in resistance to superinfection was lost if the interval between the initial and challenging doses was extended beyond 15 days.

McCoy (1931b), Bachman and Oliver-Gonzales (1936), Roth (1939), Culbertson (1942a), and Fischtal (1943) were able to induce resistance to challenge with Trichinella spiralis. This protection enabled these animals to withstand challenge doses that proved fatal to the controls. Culbertson and Fischtal indicate that this immunity is primarily directed against the intestinal phase of the infection. Fischtal's studies note that 14 days were adequate for the development of immunity.

Intestinal infections involving either male or female trichina larvae conferred a persistent, pronounced immunity to reinfection, indicating a true acquired immunity established by the intestinal phase of the life cycle (Roth, 1943).

Sarles and Stoll (1935) infected cats with Toxocara cati and noted a uniformly high degree of resistance to superinfection. The reaction of the host was directed to both the migrating larvae and upon worms completing migration to the intestine. Toxocara canis infections in rabbits and dogs produced an immunity which was directed against the migrating larvae and prevented the development of the larvae beyond the second stage (Fernando, 1968a, 1968b).

Infections with one or more doses of the larvae of Trichostrongylus calcaratus, Strongyloides ratti, Capillaria hepatica, Litomosoides carinii, Angiostrongylus cantonensis and Nematospiroides dubius afforded a high degree of resistance to superinfections in mice and rats (Sarles, 1932; Sheldon, 1937; Luttermoses, 1938; Scott et al., 1958; Lim, et al., 1965; Panter, 1969a). Panter (1969b) could not detect any immune response in mice infected with Syphacia obvelata and concluded that this lack of response was due to the absence of a tissue phase in the course of the infection.

Studies on cross-protection involving several species of nematodes indicate a degree of immunity induced by heterologous infections (Louch, 1962; Crandall, et al., 1967). Sheldon (1939), however, was unable to immunize rats against Strongyloides ratti by using heat killed S. stercoralis larvae, although the two species are closely related and are morphologically and biologically similar.

Zamian (1953), Zamian and Rubel (1953), Zamian and Stoney (1954) and Zamian, et al. (1953, 1954, 1955) using trichina infected parabiosed rats examined the immune response in the uninfected twin. He and his co-workers found that the uninfected mate when separated five days after infection was more resistant when challenged 30 days later then were control rats. Uninfected mates surgically combined with immune mates were able to withstand lethal challenges that caused death in the controls. However, Boyd and Peterson (1954) noted that larvae could migrate to the uninfected twin via the common blood vessels. These latter workers united uninfected rats with those which had been infected four weeks prior to the union and then challenged the uninfected member. These experiments resulted in a

reduced worm burden, thereby confirming the transfer of immunity from the infected member.

The transmission of immunity from an infected mother to her offspring has been noted in trichina infected rats, rabbits and hamsters by Mauss (1940a) and Culbertson (1943).

Passive resistance to parasitic infections may be engendered by the injection of nonliving worm material or by the transfer of serum from infected animals. This resistance is generally less effective than resistance acquired from an active infection.

Kerr's (1938) attempts to transfer immunity to guinea pigs with immune serum from mice and dogs infected with A. caninum were inconclusive. Based on the survival rates in experimental and control animals, it was suggestive that a humoral antibody was present. Otto (1940) successfully transferred immunity with immune serum from A. caninum infected dogs. The degree of protection indicated an antibody of considerable potency.

Injections of plasma or serum from rats infected with S. ratti (Lawler, 1940), N. muris (Sarles and Taliaferro, 1936; Chandler, 1938; Sarles, 1939) T. spiralis (Culbertson, 1942b) and N. brasiliensis (Ogilvie and Jones, 1968) reduced the number of migrating and established larvae when administered to uninfected recipients. Similar results were obtained when immune serum from trichina infected rabbits was used (Culbertson and Kaplan, 1942).

Vaccination with material derived from larvae of T. spiralis (McCoy, 1935; Culbertson, 1942a; Dorin, 1946), N. muris (Chandler, 1932; Watt, 1943) and Nematospiroides dubius (van Zandt, 1962; Lueker, et al., 1968) protected animals when given challenge doses

with the larvae of these worms.

Crandall and Arean (1965) immunized mice with various antigens obtained from Ascaris suum. They found that the injections were effective in reducing the number of larvae that reached the lungs of animals given challenges, but not as effective as an active infection.

Ogilvie and Jones (1968) were able to transfer immunity to uninfected recipients with peritoneal, lymph and spleen cells and immune serum from rats infected with N. brasiliensis.

Bachman and Oliver-Gonzales (1936) were unable to engender immunity with intraperitoneal injections of dried and powdered T. spiralis larvae, nor were Bachman and Oliver-Gonzales (1936) and Avera, et al. (1946) successful in producing resistance to T. spiralis infections by feeding powdered larvae or immune serum.

Trematoda. Acquired resistance to schistosomes does not exhibit the same degree of protection as previously noted in nematode infections. Studies indicate a tolerance to subsequent infections as opposed to conferring protection to the infected animals (Standen, 1949; Stirewalt, 1951, 1953; Oliver and Schneiderman, 1953). Cheever, et al. (1965) indicated there is little evidence that mice develop immunity to Schistosoma mansoni infections. Naimark, et al. (1960) found that single infections with Schistosoma mansoni in monkeys conferred a degree of immunity that could be demonstrated two years later when challenged with cercariae of this worm.

Resistance to challenge with Schistosoma mansoni cercariae varied from good to none after ova and adults of this species had been transferred to the portal system of monkeys (Edwards, et al., 1967). Crandall and Hunter's (1961) studies gave evidence that the presence

of Schistosoma mansoni ova as a result of natural infection or by injection may increase resistance to a homologous challenge with cercariae.

Mice infected with Schistosomatium douthitti, and then given drugs to terminate the infection, were found refractory to subsequent challenges (Kagan, 1951; Kagan and Lee, 1953). Lang (1967) was able to demonstrate an acquired immunity in rabbits to challenge with Fasciola hepatica after two prior exposures to metacercariae.

Attempts to passively transfer immunity to Schistosoma mansoni with immune serum have met with little success (Stirewalt and Evans, 1953; Levine and Kagan, 1960; Weinmann and Hunter, 1961; Ogilvie, et al., 1966). Meisenhelder, et al. (1960) used massive transfusions of immune blood which failed to exert any effect on immature or mature Schistosoma mansoni worms in monkeys.

It has been shown that vaccinations with materials derived from the life stages of schistosomes have a slightly higher protective quality than immune serum (Levine and Kagan, 1960; Sadun and Lin, 1959; Watt, 1949).

Kerr and Petkovich (1935) immunized rabbits with dried F. hepatica worm material and when these animals were subjected to re-infection with metacercariae of this species they found fewer worms than in controls. Isolated F. hepatica protein antigens were used to immunize rabbits by Urquhart, et al. (1954). Action of the immunity retarded the development, but did not reduce the number of worms established. Immunity as the result of the transfer of peritoneal exudate cells from F. hepatica infected rabbits to uninfected recipients enhanced the speed of the response to the developing

parasites (Lang, et al., 1967). Attempts by So (1960) to immunize against Paragonimus ohiari with worm extracts were unsuccessful.

Two studies on cross-protection involving nematode and schistosome infections gave completely opposite results. Hunter, et al. (1967) immunized mice with N. brasiliensis larvae and later challenged with cercariae of Schistosoma mansoni. This resulted in a significantly lower recovery of schistosomes when compared with the controls. Similar results were not obtained with reciprocal experiments. Weinmann (1960) recovered more adult Schistosoma mansoni after challenge in mice that had been previously infected with T. spiralis. He concluded that prior infection with T. spiralis makes the mice more susceptible to infections with Schistosoma mansoni. Ascaris suum infections increased resistance to S. douthitti challenges, while Schistosoma mansoni infections enhanced resistance to A. suum infection (Crandall, et al., 1966).

Cestoda. Prior infections with cestodes afford a pronounced immunity to reinfection. Larval cestode infections of Monezia expansa in sheep (Seddon, 1931; Stoll, 1935), Taenia taeniaformis in rats (Miller, 1931; Miller and Massie, 1932), bovine cysticercosis (T. saginata) (Penfold, et al., 1936; Urquhart, 1961), T. hydatigena in sheep (Sweatman, 1957; Gemmell, et al., 1968), T. pisiformis in rabbits (Kerr, 1935; Leonard and Leonard, 1941) Echinococcus granulosus in sheep (Sweatman, et al., 1963) and Hymenolepis spp. in mice (Shorb, 1933; Hunninen, 1935; Hearin, 1941; Heyneman, 1962a) have been shown to induce immunity to reinfection with these species.

Leonard and Leonard (1941), Bailey (1951) and Gemmell (1962) conclude that there are two phases of response on the part of the host:

an "early" or intestinal phase directed at the penetrating larvae and a "late" or parenetric phase acting at the site of predilection. Noda (1956) examined the dual antibody response in H. nana infections and concluded that antibodies were directed against both the tissue phase and the lumen phase. To further test the hypothesis of antibody response to the lumen phase, Coleman and deSa (1964) implanted adult H. nana to bypass the tissue phase and were able to detect antibody(s).

Weinmann and Rothman (1967) found that stress hindered the development of acquired resistance and increased the rates of re-infection in mice with a well established acquired immunity.

Cross-protection to H. nana by infections with nematodes and other cyclophyllidean cestodes have been attempted (Larsh and Campbell, 1952; Heyneman, 1962b; Weinmann, 1964). Hymenolepis nana challenges superimposed on existing nematode infections resulted in no reduction in the establishment of this cestode; however, mice with trichinosis were more susceptible. The resistance resulting from these infections was not comparable with that imparted by prior exposures to H. nana.

Cestodes which lack a tissue phase have been thought until recently unable to elicite an immune response. Chandler (1939) concluded that in rats previously infected with H. diminuta there was no effect on the subsequent challenges in relation to the establishment of new infections. He suggested that "premunition" in tapeworm infections is due to crowding rather than to immunity in the ordinary sense. Miller (1932) was unable to demonstrate premunition in cats infected with T. taeniaformis as a factor in eliminating existing infections or protecting against subsequent challenges.

Weinmann (1966) was unable to reinfect mice previously infected

with H. diminuta and subsequently treated with antihelminthic drugs. He also found that prior H. citelli infections conferred a lesser degree of protection to reinfection with H. diminuta.

Tan and Jones (1967, 1968) infected mice with irradiated H. microstoma adults and cysticercoids. These were eliminated and when mice were reexposed to normal adults and cysticercoids there were significantly fewer and shorter worms present than in controls.

Infections with some pseudophylidean cestodes are able to induce an immunity of varying proportions. Tarassov (1937) experimentally infected himself with D. latum and found that a temporary immunity was established. After three subsequent feedings he was unable to establish a patent infection. He considered this immunity as transient although it lasted for about three years. Joyeux and Baer (1939) noted that some pseudophyllidean cestodes induce in the host a protection against reinfection which may last for some time after the loss of the parasite; D. latum for period up to a year or longer, Ligula intestinalis for 20 to 30 days, while infections with D. erinacei afford no immunity.

Mueller (1969), as previously noted, examined the possibility of acquired immunity in mice previously infected with plerocercoids of S. mansonoides. Experimental mice which had been injected with four plerocercoids, and controls, were challenged with an unknown number of procercoids. The immunized mice survived infecting doses which killed four of five controls.

Passive immunity as a result of injections of material derived from various cestodes has been demonstrated. Injections of worm material derived from H. nana (Larsh, 1944), Cysticercus fasciolaris

into rats (Miller, 1930; Kerr, 1935), C. pisiformis into rabbits (Miller and Kerr, 1932) and E. granulosus (Turner, et al., 1933, 1936, 1937) into dogs and sheep have reduced the degree of parasitism when exposed to initial challenges with these worms. Chandler (1940) was unable to induce immunity to H. diminuta with oral feeding of worm material, implantation of worms in the body cavity or a series of injections of saline suspensions of dried and powdered worms.

Immunization with injections of hexacanth embryos of T. hydatigena or T. ovis (Gemmell, 1964, 1965; Blundell, et al., 1968) protected sheep when given challenges with eggs of these species. These injections also stimulated passively transferable humoral factors that provided some protection against challenges with the eggs of either species (Blundell, et al., 1968). Serum with similar protective properties have been obtained from T. taeniaformis and T. pisiformis infected animals (Miller and Gardiner, 1932; Miller, 1934; Leonard, 1939, 1940). Froyd (1964), however, was unable to induce immunity with immune serum from T. saginata infected cattle when injected into recipients challenged with eggs of this species.

Cross-protection experiments with the larvae and adults of T. taeniaformis and various other cestodes were performed by Miller (1935). Injections with powdered worm material of H. nana and T. saginata, conferred resistance of statistical significance, however, similar injections with D. latum and Dipylidium caninum afforded no resistance to T. taeniaformis. Adults of this worm implanted into the body cavity engendered a high degree of resistance, while larval implants conferred complete protection to reinfection.

The transmission of immunity from an infected mother to her

offspring has been noted in H. nana (Larsh, 1942) and larval T. taeniaformis infections (Miller, 1935b).

In summation of the preceding investigations, acquired immunity as a product of previous or existing infections with nematodes, trematodes and cestodes initiate immunological responses that are more quickly recalled when new infections are imposed on the host. Once exposed to antigenic stimuli, immune responses are more quickly engendered with additional antigenic exposure. The antibodies produced are protective as reflected by the reduction in degree of parasitism.

Passive immunity obtained by injections of material derived from the parasites induces the same reaction. The difference in effectiveness is due to the decreased amounts of antigen which are available for protective antibody formation. Active infections afford a continuing supply of these substances.

Passive immunity conferred by the transfer of immune serum is due to the presence of protective antibodies in that serum. These short lived antibodies are more readily available to affect the invading parasite. The antibodies do not induce antibody production, but inhibit the development of the parasite until immune responses of the recipient can be stimulated by the presence of the parasite.

In Vitro Action of Immune Serum on the Life Stages of Helminths

It has been previously noted that injections of immune serum can have an adverse effect on developing or patent parasitic infections. This would infer that immunity so derived would be due in part to the presence of antibodies in the serum.

Nematoda. Larvae of *T. spiralis* (Mauss, 1940) and *A. caninum* (Otto, 1940) incubated in immune serum were reduced in their ability to infect animals. However, Roth (1941) could demonstrate no deleterious effect of immune serum on trichina larvae and the infectivity of *N. muris* larvae was reduced only after prolonged incubation in immune serum (Thorson, 1954).

Precipitate formation at the openings of body orfices and in the gut of nematodes has been observed. Such precipitates have been noted to form on the larvae and adults of *T. spiralis* (Oliver-Gonzales, 1940, 1941) and *Heterakis spumosa* (Smith, 1953), the larvae of *N. muris* (Sarles, 1938; Thorson, 1953), *A caninum* (Otto, 1939), *Necator americanus* (Sheldon and Groover, 1942; Otto, et al., 1942), *T. canis*, *T. cati* and *T. leonina* (Olson, 1960; Hogarth-Scott, 1966), *A. lumbricoides* (Oliver-Gonzales, 1943), *N. dubius* (Panter, 1969b) and *L. carinii* (Scott, 1952). Sarles and Taliaferro (1936) prior to these studies had noted precipitates in and around the gut of *N. muris* larvae *in situ*.

Similar precipitate formation was noted on the exsheathed larvae of *Haemonchus contortus*, *Ostertagia circumcincta*, *Trichostrongylus colubriformis*, *Cooperia curticei*, *Nematodirus* spp., *Oesophagostomum colubianum*, *Chabertina ovina* and *Trichuris ovis* when

incubated in serum from sheep concurrently infected with these species of nematodes (Hawkins and Cole, 1944). No precipitate formation was noted on the ensheathed larvae of these species.

Oliver-Gonzales (1941) stated that immune serum contains antibodies directed toward the different life stages of parasites. Lawler's (1940) studies indicated to him that these sera owed some of their protective qualities to the ability to interfere with the metabolism of the larvae by precipitating their metabolic products.

Trematoda. Larval stages of Schistosoma mansoni are adversely effected when placed in serum from schistosome infected animals. Papirmeister and Bang (1948) and Standen (1952) observed enveloping precipitates on cercariae of Schistosoma mansoni when placed in immune serum, however, Stirewalt and Evans (1955) noted this formation was sporadic and occurred in both normal and immune serum. Cercum-cercarial membrane formation (Levine and Kagan, 1954; Kagan, 1955) and cercarial agglutination (Kagan and Levine, 1956; Senterfit, 1958a) were observed when cercariae were incubated in immune serum. It has also been noted that miracidia of Schistosoma mansoni are immobilized when placed in the serum of infected animals (Kagan, 1955; Senterfit, 1953, 1958a, 1958b; Jamuar and Lewert, 1967).

Sera from cats infected with P. kellicotti for 210 days did not immobilize miracidia or cercariae or agglutinate cercariae of this species (Seed, et al., 1966).

Cestoda. Silverman (1955) noted that immune serum caused precipitate and membrane formation on and lysis of taeniid hexacanth embryos. Precipitate formation occurred inside the outer shell membrane of intact eggs of T. saginata and T. pisiformis (Silverman,

1955) and H. nana (Heyneman and Welsh, 1959) when they were placed in the respective immune serum. The cysticercoids of T. taeniaformis when placed in immune rat serum (Chen, 1950) and the cysticercoids and adults of H. nana incubated in immune rabbit serum (Heyneman and Welsh, 1959) were affected morphologically, enveloped by precipitates and/or killed.

Weinmann (1966) found immune mouse serum to have no adverse effect on adult worms of H. nana. However, worms of this species incubated in normal and immune mucosal extracts were markedly affected. The rate and degree of action of these extracts differed greatly. All worms incubated in the immune extracts were dark and granular in appearance, whereas, about five per cent of the worms in the normal extract exhibited this appearance. He also tested the infectivity of immature worms incubated in the immune mucosal extracts and found that none was able to develop a patent infection.

Mueller (1961) placed plerocercoids of S. mansonoides in serum from chronically infected mice and noted the formation of a precipitate along the entire length of the worms. The area of heaviest precipitate formation was at the opening of the excretory ducts.

The general deleterious effect of immune serum on the life stages of a parasite is indicative of one mode of protection manifested by the host. The mechanisms engendered in sera are apparently directed against the activities of the parasite causing irreversible physiological and morphological changes.

Changes in Serum Proteins

Parasitic infections are often reflected by the changes in the total serum protein and the fractions of which it is composed. One of these changes is an increase in the globulins which may indicate the formation of antibodies to the infectious agent.

Nematoda. Shumard, et al. (1957) and Kuttler and Marble (1960) found that lambs concurrently infected with a variety of nematode species exhibited decreased values of total serum proteins. These changes were reflected by decreased albumin and increased globulin fractions. Calves infected with T. axei (Leland, et al., 1959) and O. ostertagi (Anderson, et al., 1960) and dogs infected with A. caninum (Tomado, 1963b) were shown to have similar protein changes.

Rabbits experimentally infected with T. spiralis exhibited decreases in serum albumin and elevated globulins (Mauss, 1941; Wright and Oliver-Gonzales, 1943; Kagan and Goodchild, 1961), while, Linicome and Fergussen (1964) observed the same pattern in trichina infected rats. Mauss (1941) and Wright and Oliver-Gonzales (1943) further noted that antibodies were formed in the gamma globulin fraction.

Infections in cattle with Dictyocaulus viviparous (Weber, 1957; Djafar, et al., 1960), sheep with S. papillosus (Turner and Wilson, 1960) and T. axei (Leland, et al., 1960) rats with Amplicaecum robertsi (Dobson, 1968), and calves with several other species of nematodes (Herlich, 1962) resulted in hypoproteinemia and hypergammaglobulinemia. Leland, et al. (1961) and Herlich (1962) also noted that there was a decrease in the total proteins as a result of some nematode infections.

Beta globulins have been implicated in antibody formation. Greatly increased beta globulin values have been noted in infections with N. muris in rats (Leland, et al., 1955) and H. contortus and O. columbianum in sheep (Mould and Silverman, 1959; Dobson, 1965). Dobson (1965) analyzed the intestinal mucus from animals in his studies and found it to contain large quantities of beta globulin.

Experimental strongyloidiasis in sheep and goats (Turner, 1959) L. carinii infections in rats (Zein-Eldin and Scott, 1961) and Dirofilaria immitis infection in dogs (Tomoda, 1962) induced higher levels of the beta and gamma globulins. Beta globulin increases occurred during the early migration and development, while the gamma globulins attained higher levels at the time infections became patent.

In contrast to the previous studies, rats infected with T. spiralis (Kagan and Goodchild, 1961) and calves infected with H. placei (Anderson, et al., 1960) exhibited no change in any of the serum proteins.

Trematoda. Schistosoma mansoni infections in mice (Evans, et al., 1955; Evans and Stirewalt, 1957, 1958; Kagan and Goodchild, 1961; Sadun and Williams, 1966; Hillyer and Frick, 1967a), in hamsters (Evans, et al., 1955; Evans and Stirewalt, 1958; Kagan and Goodchild, 1961), in cattle (Tomoda, 1963) in monkeys (Smithers and Walker, 1961; Hillyer and Frick, 1967b) and in humans (Kagan and Goodchild, 1961) produced rises in the beta and gamma globulins and total serum proteins, while albumin levels were decreased. Similar serum protein changes were noted in rats and cats infected with P. kellicotti (Kruidenier and Katoh, 1959) and rats infected with P. miyazakii (Tada, 1967).

Elevated gamma globulins and depressed albumin concentrations were noted in guinea pigs (Kagan and Goodchild, 1961) and humans (Ramirez, et al., 1961) infected with Schistosoma mansoni, in rabbits (Sadun and Walton, 1958; Tomoda, 1963), in dogs (Tomoda, 1963) and humans Sadun and Walton, 1958) infected with S. japonicum and cattle infected with F. gigantica (Weinbren and Coyle, 1960).

No set pattern of protein changes were observed in rabbits and no change in rats infected with Schistosoma mansoni (Kagan and Goodchild, 1961).

Cestoda. Kraut (1956) analyzed the serum protein changes of rats experimentally infected and passively immunized with C. fasciolaris. Rats heavily infected, 66 cysts or more, exhibited significantly increased levels of alpha, beta and gamma globulins, with decreased amounts of albumin. Rats infected with fewer cysts than those heavily infected, showed significant changes only in total proteins and the beta globulins. No significant differences in serum components were obtained in the passively immunized rats. Noda (1956) in a similar study with H. nana, observed the greatest globulin increase in the gamma fraction, with the beta rises being less consistent. He also found the same pattern in passively immunized mice.

Sera from moose, hogs, cattle and man infected with E. granulosus and serum from rats infected with E. multilocularis all had increased amounts of gamma globulin with depressed albumin levels (Goodchild and Kagan, 1961; Kagan, 1963), while sheep infected with E. granulosus showed increases in the beta and gamma fractions.

An electrophoretic comparison of hydatid cyst fluid and sera from hydatid infected animals revealed very similar patterns

(Goodchild and Kagan, 1961). On the basis of these similar patterns, they concluded that serum albumin and globulins occur in the cyst fluid but in greatly reduced amounts.

Sadun, et al. (1965) examined the biochemical changes in mice infected with the plerocercoids of S. mansonoides. Their studies revealed no alterations in the serum protein components. A slight reduction in total proteins was attributed to dilution factors.

The increased concentrations of beta and gamma globulins as a result of helminthic infections parallels similar changes that occur in bacterial or viral diseases. These changes are the result of antibody formation. The beta globulins are characterized with cell sensitizing antibodies, while gamma globulins are associated with precipitating antibodies. Dramatic decreases in albumin are thought to be caused by damage to the liver in some helminthic infections, while less significant decreases may be in response to elevated globulins to maintain a relatively constant osmotic concentrations.

Antigens

Attempts have been made to characterize the antigenic components of parasites. Excretory-secretory antigens have been obtained from the culture or incubation of parasites in various media and solutions. Somatic antigens are derived from extracts of isolated tissues or whole worms. These antigens are used to immunize animals to test their effectiveness in altering the course of challenges with a parasite. Various techniques have been used to bring together antibodies and antigens so that visible precipitates are formed. These precipitates indicate the possible number of antibody-antigen

systems that may be present.

Nematoda. Excretory-secretory (ES) antigens prepared from T. spiralis larvae (Campbell, 1955) and adults (Chipman, 1957), N. muris larvae (Thorson, 1951) and the larvae of D. viviparous, T. colubriformis and S. pappillous (Silverman, et al., 1962) were used to immunize recipients that were challenged with the larvae of these species. These ES antigen injections resulted in a reduced number of worms established after challenge.

Tanner (1963) found that the major antigens of T. spiralis larvae are mucoproteins, while Mills and Kent (1965) later identified the ES antigens as protein in nature, complexed by polysaccharides. Dusanic (1966) notes that the secretion of lactic dehydrogenase during T. spiralis infections stimulates production of specific antibodies in rabbits.

Rabbits immunized with ES or somatic antigens gave results that indicate the two antigens are not entirely distinct and that a large degree of cross-reaction exists (Sadun and Norman, 1957).

Injections of somatic antigens from T. spiralis larvae failed to produce demonstrable immunity, however, cuticular antigens proved to be immunologically functional (Moore, 1965). He concluded that the cuticles shed by molting larvae contribute to the total immunity in infected mice.

Melcher (1943) identified acid soluble protein fractions of T. spiralis which had three electrophoretic mobilities, while Labzoffsky, et al. (1959) isolated seven antigenic fractions.

Agar diffusion and immunoelectrophoresis studies have shown a minimum of three, to a maximum of 13 precipitating antibody-antigen

systems in serum from animals immunized or infected with T. spiralis larvae (Kagan and Bargi, 1956; Olson, et al., 1960; Tanner and Gregory, 1961; Sharp and Olson, 1962; Tanner, 1963; Oliver-Gonzales, 1963; Oliver-Gonzales and DeSala, 1963; Gadea, et al., 1967; Castro and Fairbairn, 1969). One to three systems have been identified with adult trichina antigen (Oliver-Gonzales, 1963; Oliver-Gonzales and DeSala, 1963; Gadea, et al., 1967). Similar studies with T. canis (Sharp and Olson, 1962; Huntley and Moreland, 1963) produced three to seven precipitating bands, however, a total of 40 were reported by Jeska (1967) for this species. Excretory-secretory antigens of infective H. contortus larvae showed serological activity forming one precipitin line with immune sheep serum and two to three lines with immune rabbit serum (Ozerol and Silverman, 1969).

Trematoda. Sadun, et al., (1964) found that antigens derived from eggs, adults and ES antigens from adults of Schistosoma mansoni, have distinct humoral activity. Agar diffusion studies by these investigators indicated that some of the antibody-antigen systems are specific to the particular life stage, however, some are common to two or three of these stages.

A total of eight to 13 precipitating bands with egg antigens (Sadun, et al., 1965; Hillyer and Frick, 1967a, 1967b), four to 12 precipitin bands with cercarial antigens (Kent, 1963; Kronman, 1965; Sadun, et al., 1965; Hillyer and Frick, 1967a, 1967b) and three to 11 precipitin bands with adult antigens was determined by gel diffusion and immunoelectrophoresis studies of Schistosoma mansoni (Sadun, et al., 1965; Damian, 1966; Hillyer and Frick, 1967a, 1967b).

Serum from animals infected with P. westermani (Swada, et al.,

1964a; Yogore, et al., 1965) formed two to five precipitating lines when matched with antigen of this parasite. Tada (1967) detected a maximum of six precipitating systems in sera from rats infected with P. miyazakii.

Cestoda. Gel diffusion and immunoelectrophoresis studies with fluid obtained from E. multiloccularis cysts produced one to four precipitin lines when matched with immune serum and fluid from E. granulosus cysts matched with immune serum resulted in the formation of two to eight lines (Kagan, et al., 1960; Kagan and Norman, 1963). Antigens prepared from the cysts of E. granulosus and E. multiloccularis produced 23 and 27 precipitating systems with their respective immune sera (Kagan and Norman, 1961).

Serum from rabbits immunized with whole worm antigens of H. nana (Coleman and Fortnoy, 1961) produced four precipitin lines when matched with this antigen. Similarly, immunized rabbits with M. expansa formed one precipitin line with gel diffusion and three lines with immunoelectrophoresis (Duroe, 1967).

It is evident from the preceding investigations that antigens derived from helminths are complex substances and their analysis incompletely known. It appears that the principal antigens are the metabolic products, although the antigenic nature of somatic tissues or extracts of these tissues has been demonstrated. It is problematical that some of the derived somatic materials would be available for antibody production during active infections.

The formation of immune precipitates in gels are indicative of the minimum number of antibody-antigen complexes. Optimal concentrations of antigens and antibodies are necessary for the full expression

of all precipitating systems. High dilutions of either factor may not provide adequate materials for precipitates to form, whereas, excessive quantities of antigen may inhibit these reactions.

Diagnostic Tests for Helminth Infections

Various tests are used for the diagnosis of parasitic diseases. Among the most frequently employed are the intradermal and precipitin tests. Other tests utilized are hemagglutination, flocculation and complement fixation.

Intradermal test

Nematoda. The use of the intradermal test for the diagnosis of nematode infections has been used for many years, but the results from these tests have not been consistent.

Investigators have attempted to use the skin test for the detection of trichinosis in marketable swine. Studies by Spindler and Cross (1939) and Spindler, et al. (1941) have shown a high percentage of positive skin reactions in swine not infected with trichinosis. Soulsby (1957) noted a high incidence of cross-reaction in ascarid infected pigs when skin tested for trichina infections. Augustine and Theiles (1932), however, considered the intradermal test more reliable than muscle examination in detecting trichina infected pigs.

The intradermal test for the detection of human trichinosis was considered very reliable by McCoy, et al. (1933), Spinks and Augustine (1935), McNaught, et al. (1941) and Lamb, et al. (1964). Arbesman, et al. (1942), however, stated that high percentages of positive reactions in normal and allergic individuals limits the skin test for trichinosis as a diagnostic procedure.

Hunter, et al. (1945) Oliver-Gonzales and Hernandez-Morales (1945) and Warren, et al. (1946) noted that antigens obtained from the microfilariae of Wuchereria bancrofti were very specific for use in intradermal tests for the detection of Bancroft's filariasis.

The difficulty of obtaining antigens for intradermal tests for the diagnosis of human filariasis, has necessitated the use of antigens derived from other species of filarial worms.

Antigens from the larvae of D. immitis (Bozicevich and Hutter, 1944; King, 1944; Alhadeff, 1955; Warren, et al., 1946; Oliver-Gonzales and Hernandez-Morales, 1945), L. carinii (Culbertson, et al., 1944b; Oliver-Gonzales and Hernandez-Morales, 1945; Wharton and Stelma, 1946) and Seteria cervi (Ridley and Scott, 1961) have been used with some success for detecting infections with W. bancrofti. Similar studies resulted in a high percentage of positive skin reactions in both infected patients and controls when these antigens were used (Ata, et al., 1967). Also individuals with intestinal nematode infections had a high rate of positive skin reactions when tested with these antigens (Oliver-Gonzales and Hernandez, 1945).

Augustine and L'herisson (1946) concluded that non-human filarial worms can influence the serologic reactions in man. They also stated that many unexplained false positive reactions following the use of animal filarid antigen may be due to immune responses produced by the filarid larvae of animals.

Antigens of D. immitis (Wright and Murdock, 1944; Bozicevich, et al., 1947), L. carinii (Culbertson, et al., 1944a; Bozicevich, et al., 1947), S. equina (Bozicevich, et al., 1947) and Onchocerca volvulus (Bozicevich, et al., 1947) have been used for the detection

of onchocerciasis and loaiasis.

Trematoda. Pratt and Oliver-Gonzales (1947) and Gazzinelli (1965) found intradermal tests with cercarial antigens of Schistosoma mansoni effective in the detection of infections with this parasite. However, intradermal tests in patients with unequivocal schistosome infections proved less accurate than other tests (Anderson and Naimark, 1960). Consistent results were obtained with the skin test for paragonamiasis (Richie, et al., 1951; Sawada, et al., 1964b) and clonorchiasis (Sadun, et al., 1959).

The presence of other intestinal parasites in humans with schistosomiasis (Ata, et al., 1967) and persons with a past history of schistosome dermatitis had a higher incidence of positive skin reactions when tested with Schistosoma mansoni antigens (Moore, et al., 1968).

Antigens derived from another species of trematode were used by Culbertson and Rose (1942). They skin tested three patients with Schistosoma mansoni infections and detected strong and immediate reactions to intradermal injections of Pneumoneces medioplexus.

Cestoda. Rabbits infected with the larval form of T. serrata exhibited a high percentage of positive skin reactions when tested with larval and adult antigens (Wharton, 1931). Cross-reactions to cestode infection have been noted when antigens derived from other cestodes are used for skin tests (Rose and Culbertson, 1939; Culbertson and Rose, 1941).

Bensted and Atkinson (1955) obtained results using the intradermal tests in patients known to be infected with E. granulosus. However, Magath (1959) and Garabedian, et al. (1959) were able to show

a consistent correlation between diagnosis and incidence of hydatid infection with skin tests.

Mueller and Coulston (1941) experimentally infected themselves with the plerocercoids of S. mansonoides. As much as a year later positive skin tests were obtained.

Precipitin test

Nematoda. The result of precipitin tests, which correlated with the findings of other tests, have been shown to be of value in the diagnosis of trichinosis (Spink and Augustine, 1935; McNaught, et al., 1941; Hendricks, 1950). Oliver-Gonzales and Bercovitz (1944) obtained a low percentage of positive results in patients known to be infected with filariasis. Bachman, et al. (1934) and Gould (1943) cautioned that non-specific reactions with the precipitin test must be considered when it is used, but this test possesses a high degree of specificity.

Trematoda. Precipitin test involving schistosome infections have been conducted by incubation of life stages in immune serum. The results have been noted in a previous section.

Cestoda. As with the trematodes, precipitin studies with cestodes have mostly involved in vitro action in immune serum. Dennis (1937) used a concentrated antigen prepared from hydatid cysts to perform precipitin tests. The results of his studies proved accurate in all animals tested. Biagi and Tay (1958) found antigen from C. cellulosae sensitive in the detection of human and swine cysticercosis when employed in the precipitin test. The precipitin test was used to demonstrate the response of mice to infection of H. microstoma (Hutchins, 1961).

The inconsistent results of the intradermal and precipitin tests

cast doubt on the value of their use in the detection of helminthic infections. Kagan (1960, 1963) indicated the need for standardization of techniques and materials before these tests can be properly evaluated for diagnostic use.

MATERIALS AND METHODS

Experimental animals

Mice. Female mice (Mus musculus) between 20 and 25 grams of the Webster strain were used for active immunization and hypersensitivity studies. These mice were from a colony maintained in the departmental animal facilities or purchased for the study. The mice were kept in metal cages using San-i-Cel as litter. Food and water were available ad lib.

Rabbits. Male or female New Zealand White rabbits (Oryctolagus cuniculus) were used for the artifical immunization studies. The rabbits weighed between three and four kilograms. These animals were maintained in metal cages and food and water available ad lib.

Plerocercoids. Larvae to be used for active and artifical immunization studies were cultured from eggs obtained from cats infected in the laboratory and known to be free of prior natural infections of Spirometra. Egg samples were placed on filter paper strips with the ends immersed in water as per the modified Harada-Mori culture method (1955). Periodic checks were made to determine the degree of development. Ciliary and flame cell activity were used as criteria for readiness for hatching. When the eggs were determined ready to hatch, the filter paper strips were placed in finger bowls containing uninfected copepods from cultures maintained in the laboratory. The eggs were then gently brushed from the strips. The culture bowls were kept under constant light at room temperature. Copepods were checked at intervals to determine the development of the

procercoids. When calcareous corpuscles and the cercomer were evident, the copepods were concentrated by filtering the water through a fine screen. Aliquots of about 25 copepods were injected subcutaneously into mice. After a month, mice were necropsied and the plerocercoids removed. These larvae were used to immunize mice or prepared as antigen.

Plerocercoids obtained from snakes (Lampropeltis getulus holbrooki, Natrix sipedon confluens, N. erythrogaster flavigaster) were used for active immunization and antigen preparation in one experimental group.

Antigen

Plerocercoids to be used as antigen were removed from infected mice and washed three times in 0.85 per cent saline to remove extraneous material. The larvae were blotted on filter paper to remove excess saline, placed in vials and frozen in a dry ice-acetone solution. The contents of the vials were lyophilized for 48 hours, removed, placed in a pre-cooled dessicator containing calcium chloride and placed in a freezer for 24 hours. The vials were capped and stored under freezing conditions until used.

Lyophilized larvae for immunization were weighed as needed, placed in saline and disrupted by sonic vibrations for 10 minutes. The resulting mixture was combined with equal amounts of Fruend's complete adjuvant and emulsified.

A stock antigen solution for gel diffusion studies and intradermal tests was prepared by sonicating 500 mg of lyophilized larvae in five milliliters of 0.85 per cent saline. The mixture was centrifuged, the supernatant removed, frozen and stored in aliquots

of 0.5 milliliters. The solutions contained 0.1 milligrams of larval antigen per 0.01 milliliter.

Blood samples

Approximately 0.1 milliliter of blood was taken from each mouse in a group of inserting a capillary pipette through the orbital sinus membrane. When the sinus is punctured, blood enters the tube by its own pressure. Post infection samples were taken at three, 12 and 24 hours and at four day intervals thereafter. Samples were taken for 12 weeks. Blood from mice in a test group were pooled for each sampling period.

Blood samples were obtained from rabbits by bleeding from the middle ear vein. A shallow incision was made at right angles into the ear vein. A centrifuge tube was pressed against the ear close to the incision and 10 milliliters of blood allowed to flow into the tube. Afterwards pressure was applied to the cut to stop the flow and allow the blood to clot.

Blood collected from mice and rabbits was allowed to clot at room temperature for three hours. The clot was ringed and the tube placed in the refrigerator overnight to allow clot contraction. The samples were centrifuged for 30 minutes at 3000 revolutions per minute. The serum was removed, frozen and stored until used.

Immunization procedures

Mice.

Active immunization. Three groups of 15 mice were fed plerocercoids of *Spirometra mansoni*, *S. urichi* and *S. mansonoides* "A". One group of ten mice was similarly infected with the plerocercoids of *S. mansonoides* "B" obtained from snakes. Mice were lightly

anesthesized with ether and ten scolices were placed in the mouth. Small amounts of water were administered following the placement of the scolices to enhance swallowing. Each mouse was held in an individual container to determine if any scolices were lost. None was observed lost from any mice.

Two groups of fifteen mice were used as controls.

Artificial immunization. Ten experimental and five control mice were used for each hypersensitivity test. Experimental mice were immunized with two intraperitoneal injection of saline antigen-adjuvant mixtures one week apart. A total of 20 milligrams of lyophilized larval antigen was used. Controls received two sham injections of saline-adjuvant mixture.

Rabbits.

Artificial immunization. Each rabbit received two intramuscular saline antigen-adjuvant immunizing injections. Prior to immunization each rabbit was bled from the middle ear vein. An initial injection equal to 50 milligrams was administered. Rabbits were prebled at four weeks and the second immunizing dose was given. Six weeks after the initial immunizing injection the rabbits were bled for the final time. Attempts were made to give intravenous booster injections, but they induced anaphylactic shock. Another rabbit was given saline-adjuvant injections to serve as a control.

Serum protein determination

Total serum protein concentrations were determined using the Biuret reaction. One tenth milliliter of serum was diluted with 3.0 milliliters of 0.85 per cent saline. One milliliter of the solution was mixed with 2.0 milliliter of 0.85 per cent saline and 3.0

milliliters of Biuret reagent. The mixture was incubated at room temperature for 30 minutes. Readings were made with a Bausch and Lomb Spectronic 20 at 520 millimicrons to wavelength and compared against a standard protein concentration curve constructed using bovine serum albumin.

Zone electrophoresis was conducted on the sera to determine concentration of serum protein components. A Beckman Durrum cell was used with a barbital buffer, pH 8.6 and ionicity of 0.075. Power was supplied at 2.5 milliamperes and 110 volts d.c. by a Heathkit Variable Voltage D.C. Power Supply.

Six lambda of serum were applied to Schleicher and Schuell 2043A filter paper strips and current applied for 16 hours. The strips were oven dried at 120° Centigrade for 30 minutes. Strips were prerinsed in absolute methanol for six minutes, strained for 30 minutes in alcholic bromphenol blue and rinsed three times in 5 per cent acetic acid for six minutes each. The strips were then dried for 15 minutes at 120° Centigrade and developed in ammonium hydroxide vapors for 30 minutes.

A Beckman-Spinco Analytrol was used for strip analysis. The peaks for each fraction on the tracing pattern were marked off with vertical lines and the number of divisions on the integrator line beneath each peak was counted. Relative concentrations for each fraction were calculated against the sum of the total marks on the integrator line.

Gel diffusion

The micro-Ouchtolony double gel diffusion technique was used to detect precipitating antibodies. Three milliliters of one per cent

Difco Special Agar - Noble were pipetted onto a slide giving an area of 25 millimeters by 45 millimeters and a depth of three millimeters. Slides were refrigerated overnight.

A plexiglas template was constructed with a central and four surrounding holes. The hole in each quadrant was five millimeters from the central hole. All holes were three millimeters in diameter. The template was placed over the slide and a sharpened metal tube used to cut wells in the agar. The agar plugs were removed with a probe, each well filled with melted agar and quickly aspirated to seal them at the base.

One slide was used for each serum sample from the experimental and control animals. The central well was filled with serum and two of the surrounding wells filled with antigen. The other wells were filled with 0.85 per cent saline to serve as controls. The wells of each slide were filled three times to assure adequate materials to diffuse into the agar and react to form precipitin lines. The slides were stored at room temperature in a moist atmosphere. Observations were made at 24, 48 and 72 hours.

Absorption studies

One tenth milliliter of immune serum was mixed with 20 milligrams of powdered lyophilized larvae and allowed to stand for 30 minutes. The resulting mixture was centrifuged to remove particulate matter and the supernatant matched with the homologous antigen on a gel diffusion slide. One slide was used for each serum sample obtained during the study period.

Hypersensitivity test

Hair was removed from the dorsal surface of experimental and

control mice 24 hours prior to skin testing with an Oster small animal clipper. The mice were lightly anesthized with ether and immobilized on a restraining board. A 27 gauge needle and a tuberculin syringe were used to inject 0.01 milliliters of antigen solution intradermally. Observations were made at three, 12, 24, 48 and 72 hours after the injection.

Solutions and Media

Borate buffer

6.184 grams - Boric Acid
9.536 grams - Sodium Borate
4.384 grams - Sodium Chloride

Above ingredients dissolved in 1000 milliliters distilled water and the pH adjusted to 8.4 or 8.5 with Hydrochloric Acid or Sodium Hydroxide.

0.15 molar NaCl

8.77 grams - Sodium Chloride

Sodium Chloride dissolved in 1000 milliliters distilled water.

0.85 per cent NaCl

8.5 grams - Sodium Chloride

Sodium Chloride dissolved in 991.5 milliliters distilled water.

Biuret reagent

45.0 grams - Sodium Potassium Tartrate
15.0 grams - Copper Sulfate
5.0 grams - Potassium Iodide

Dissolve Sodium Potassium Tartrate in 400 milliliters 0.2N Sodium Hydroxide, add Copper Sulfate and dissolve completely. Add Potassium Iodide and make to one liter with 0.2N Sodium Hydroxide.

0.2N Sodium Hydroxide

8.0 grams - Sodium Hydroxide

Dissolve in one liter of distilled water.

1 per cent special noble agar

1.0 gram - Special Noble Agar

Dissolve agar in 5 milliliters borate buffer and 95 milliliters of 0.15 molar Sodium Chloride solution in a boiling water bath. Cool to 45° Centigrade before pipetting onto slides. One milliliter of metheriolate 1:10000 is added as a preservative.

RESULTS

Total serum protein values were determined and zone electrophoresis was performed on 160 blood samples collected from mice and rabbits during the experimental period. Concurrently, micro-gel diffusion was performed on these sera before and after absorption with their homologous antigens. Blood samples were obtained from each test group over a period of 84 days. These samples were taken initially at three, 12 and 24 hours and at four days intervals thereafter. The frequency of initial sampling was selected to determine if the "early" phase of immunity observed in some cestode infections (Campbell, 1938a, 1938b, 1938c; Hearin, 1941; Coleman and deSa, 1964; Weinmann, 1964) would be reflected in serum protein changes or the presence of precipitating antibodies in the sera from infected mice.

Average protein concentrations were determined for the 24 serum samples from each group collected during the experimental period. These concentrations are expressed in grams per 100 milliliters (grams/100 ml).

Total serum protein values for the control group average 6.00 (5.25-6.66) grams/100 ml during the test period (Figure 1).

Mice infected with Spirometra mansoni had serum protein concentrations that varied more than any other experimental group (Figure 2). Values remained within the levels previously noted for the controls (5.26-6.66) until day 36, at which time the concentrations began to increase, reaching a peak slightly in excess of 8.00 at 44 days. This level was maintained until day 52. At day 56 the values returned to

normal only to increase to the previous high level at 60 days. This elevation was maintained until day 72, after which time the concentrations dropped below the average protein levels of the controls for the remainder of the study period.

Total protein concentrations for the mice infected with S. urichi averaged 6.41 (5.61-8.19) grams/100 ml (Figure 3). An irregular protein concentration pattern was exhibited for part of the study period. An initial value of 7.20 was above the level of the controls. A decline to 5.55 was noted until day four, then the concentrations rose to the previous level at day eight. Concentrations returned to the level of the controls until day 36, when they rose to 8.19, equal to those of the Spirometra mansoni group. This level was maintained until day 48, whereafter the concentration declined to the average (6.41) for the remainder of the study period.

Mice infected with S. mansonoides "A" exhibited little variation in the total serum protein concentrations (Figure 4). The average of 6.00 (5.49-6.48) grams/100 ml was the same as that for the controls.

The protein levels for the S. mansonoides "B" group were below the average protein concentrations of the controls for most of the study period. The average value for this group was 5.60 (4.80-6.21) grams/100 ml (Figure 5). The protein concentrations remained stable at the 12 hour value of 5.55 until day 36, when it dropped to 4.80, followed by a rise to the previous level at 52 days. The concentrations for the rest of the study period were near 6.00.

Electrophoresis

Zone electrophoresis was conducted on all sera collected during the experimental period. Four protein fractions were consistently demonstrable in all sera examined. These fractions were albumin and alpha, beta and gamma globulins. The alpha-1 and alpha-2 globulins were for the most part inseparable and were considered as one fraction. Because of the variation in the total serum proteins, concentrations for the protein fractions are expressed in relative per cent of the total protein values.

Controls

Absolute values for the serum protein fractions in the control group is given in Table I and relative percentages in Table II. Albumin levels gradually declined throughout the test period, from an initial value of 80.2 to a final concentration of 69.7 (Figure 1). A level of approximately 80.0 was maintained through 44 days, after which the decline was more pronounced.

Globulin changes (Figure 1) were exemplified by a rise in all fractions; alpha, from 7.2 to 12.4 per cent; beta, 9.9 to 12.4 per cent; gamma, from 2.7 to 5.4 per cent.

Spirometra mansoni

Relative percentage values for the serum protein fractions for mice infected with Spirometra mansoni are shown in Table III and the absolute concentrations for this group in Table IV.

Albumin. Albumin changes in the sera of these mice occurred in three major peaks (Figure 2). An initial value of 67.4 was noted at three hours post infection, thereafter peaks occurred at 20, 36 and 68 days, with concentrations of 68.0, 71.0 and 67.9 respectively.

These levels were approximately equal to the initial value of 67.4. After 68 days, the quantities declined to their lowest level of about 55.0 until the termination of the study.

Alpha globulins. The alpha globulins remained relatively stable for the entire 84 days (Figure 2). Identical initial and final concentrations of 11.1 were noted.

Beta globulins. The beta globulins had an initial level of 17.2 and a terminal value of 18.9. During the experimental period, three minor rises to about 20.0 were noted at four, 24 and 80 days. These rises were approximately during the time when albumin levels were depressed (Figure 2).

Gamma globulins. The gamma globulins continually rose throughout the study period, from an initial value of 5.3, to a final concentration of 14.4, an increase of 9.1 (Figure 2). A rise to 13.0 was noted at 28 days post infection, followed by a decline to 8.1 until day 40. Values were again elevated at 44 days when a concentration of 14.0 was attained and at which time antibodies could be detected by gel diffusion.

Spirometra urichi

The relative percentage concentrations of proteins in serum from mice infected with S. urichi are shown in Table V and the absolute concentrations in Table VI.

Albumin. Albumin concentrations declined from a level of 63.5 at three hours, to 55.4 until 32 days post infection, at which time a protracted increase to 65.9 was observed 20 days later (Figure 3). The concentrations then decreased to a low value of 48.4 at 84 days.

Alpha globulins. The alpha fraction was elevated above the

initial value of 9.4 for the entire 84 days. Three peaks of about 16.5 occurred at eight, 64 and 76 days (Figure 3).

Beta globulins. Beta globulin concentrations began at 19.8, fluctuated below this level until day 16 when the values returned to the initial concentration. Concentrations again declined gradually until day 56 when another rise began. The rise continued until the termination of the study period, when a maximum value of 22.7 was noted (Figure 3).

Gamma globulins. Gamma globulin concentrations began at 7.3 and generally remained at this level until 16 days post infection, when a gradual rise began which continued until day 84 (Figure 3). A maximal level of 16.4 was noted at this time. Antibodies were detected at 40 days, eight days after a concentration of 13.5 was reached.

Spirometra mansonoides "A"

Relative percentages are given in Table VII and the absolute concentrations for serum protein fractions in sera from mice infected with S. mansonoides "A" in Table VIII.

Albumin. Serum albumin concentrations were generally lower than in any other group. Correspondingly higher levels of the globulin fractions were also noted (Figure 4). Albumin values declined from a maximum concentration of 64.8 at 12 hours, to a minimum of 43.7, 76 days post infection. The concentrations than rose to 51.0 at the termination of the study.

Alpha globulins. Elevated alpha globulins occurred between the first and last sample taken. The initial and terminal concentrations were 13.8 and 14.1 respectively (Figure 4). Maximum values of 19.7 occurred at 16 and 80 days.

Beta globulins. Beta globulin changes were reflected by a steady increase (Figure 4). Maximum concentrations of approximately 23.0 at 20, 48 and 76 days were noted after an initial value of 16.9.

Gamma globulins. The quantity of gamma globulin also increased steadily from 7.7 at three hours, to a maximum of 20.2 at 56 days (Figure 4). The total increase of 12.5 was the greatest that occurred between the initial and maximal concentrations for gamma globulins in any group. Antibodies were detected at 40 days when the concentration was 16.5.

Spirometra mansonoides "B"

Table IX shows the relative concentrations in per cent and Table X gives the absolute concentrations of proteins in sera from mice infected with S. mansonoides "B".

Albumin. Albumin concentrations varied from lows of 47.7 at eight days and 43.4 at 56 days, to highs of 66.7 at 12 and 24 hours and 65.1 at 32 days post infection (Figure 5). Values between these intervals were increasing to or declining from the initial value of 62.9.

Alpha globulins. Alpha globulin concentrations rose from the three hour value of 10.5, to 16.5 at four days. This rise was followed by a return to the initial level until 44 days post infection. After this time concentrations were elevated to maximum values of 18.3 at 60 days and 84 days post infection (Figure 5).

Beta globulins. The beta globulin concentration was 16.1 at three hours. Following this time there were two peaks that attained maximum values of 24.4 at eight days and 25.2 at 56 days (Figure 5). Values at other times approached or were maintained at the initial

level of 16.1.

Gamma globulins. An initial gamma globulin concentration of 10.5 was higher than in any other group. Thereafter values were generally below this level until 24 days post infection. Concentrations then increased gradually reaching a maximum concentration of 18.9 at 56 days (Figure 5). A slight decline to 16.2 was noted at the termination of the study. Antibodies were detected at 40 days, 12 days after a concentration of 14.8 was observed.

Passive Immunization

Total serum proteins were determined for the sera from rabbits given sham injections and those immunized against Spirometra mansoni, S. urichi and S. mansonoides "A" and "B". Absolute concentrations and percentage values of serum proteins for sham injected controls are given in Tables XI and XII and rabbits immunized against Spirometra mansoni in Tables XIII and XIV, for S. urichi in Tables XV and XVI, for S. mansonoides "A" in Tables XVII and XVIII and for S. mansonoides "B" in Tables XIX and XX.

Total serum protein concentrations exhibited little change before or after immunization or sham injections. Total protein values before and after injections for the control averaged 7.08 grams/100 ml; immunized against Spirometra mansoni,7.15 grams/100 ml; S. urichi, 7.17 grams/100 ml; S. mansonoides "A", 6.75 grams/100 ml; S. mansonoides "B", 7.21 grams/100 ml.

Pre- and post-immunization sera were subjected to electrophoresis to determine quantitative changes. Serum from the control animal was similarly treated.

Albumin concentrations in the sera from the rabbits immunized against Spirometra mansoni (Table XIII) and S. mansonoides "A" (Table XVII) and "B" (Table XIX) decreased about 0.25 (3.0 per cent) grams/100 ml at six weeks post-immunization, whereas, the albumin level in the serum from rabbits immunized against S. urichi dropped 0.77 (14.3 per cent) grams/100 ml (Table XV).

Alpha and beta globulin concentrations remained relatively constant in all serum samples.

Maximum gamma globulin increases were obtained in all immune serum samples at six weeks post immunization. Gamma globulin values for the serum from the rabbit immunized against Spirometra mansoni was 1.21 grams/100 ml (Table XIII), an increase of 4.80 per cent. A gamma globulin concentration of 1.72 grams/100 ml (Table XV) was noted in the serum from the rabbit immunized against S. urichi. This was an increase of 13.4 per cent above the initial preimmunization gamma globulin concentration. Rabbits immunized against S. mansonoides "A" (Table XVII) and "B" (Table XIX) exhibited gamma globulin concentrations of 0.19 an increase of 2.7 per cent, and 0.17, an increase of 2.0 per cent, respectively.

Average protein fraction values for the control rabbits were as follows; albumin, 4.82 (68.0 per cent) grams/100 ml; alpha globulin, 0.81 (11.4 per cent) grams/100 ml; beta globulin, 0.77 (10.8 per cent) grams/100 ml; gamma globulin, 0.68 (9.9 per cent) grams/100 ml.

Gel Diffusion

Precipitating antibodies were detected in immune mouse sera from the four experimental groups by gel diffusion. None was observed in the sera of the control group.

One precipitin band was observed at 44 days with mouse serum from the Spirometra mansoni group. The serum from the 48 day sample formed a maximum of two precipitating bands (Plate I, Fig. a) which were demonstrable with these sera from the remainder of the test period.

Sera from the mice in the S. urichi and S. mansonoides "B" (Plate I, Fig. b, d) groups formed one band when matched with their respective antigens. These bands were first evident at 40 days in both groups and were observed in all sera from these groups through 84 days.

Two precipitin bands were formed at 40 days with the serum from the S. mansonoides "A" group and could be demonstrated through the remainder of the study (Plate I, Fig. c).

Concurrently with the above tests, immune sera were absorbed with the homologous powdered lyophilized antigen and then were subjected to gel diffusion. At no time did precipitin bands form with the immune sera that had been treated in this manner.

Control mouse serum from each test day was matched against each species antigen. No precipitating bands were noted in any of these sera when subjected to gel diffusion.

Gel diffusion was employed on sera obtained from rabbits immunized against Spirometra mansoni, S. urichi and S. mansonoides "A" and "B". All sera obtained at four weeks post immunization formed

one precipitin band in agar gel. Three bands were formed with each of the immune sera obtained at six weeks post immunization (Plate II, Fig. a, b, c, d). No precipitin bands were detected with pre-immunization or control sera, nor were any bands observed after immune sera had been absorbed with their respective antigens.

Intradermal Test

There are classes of antibodies that are not detectable as precipitins. These antibodies are quickly and permanently bound to body cells and may be detected by intradermal injections of antigen into sensitized animals. A reaction occurs that is characterized by erythema, edema and induration.

Skin tests were performed on two groups of ten mice, one previously immunized against Spirometra mansoni and the other against S. urichi. Two groups of five mice served as controls. Base-line criteria for interpretation were to be established by a comparison of reactions in the experimental and control animals.

The reactions observed in the individual mice from the immunized groups were highly variable. When injected intradermally with antigen, the reactions ranged from very intense areas of edema and induration, to little response at all. Reactions in the controls also varied but in general were much less intense.

A second group of mice immunized against Spirometra mansoni was tested. The results of this test were as variable as the others. In light of these results further experiments involving intradermal tests were discontinued.

Table I. Absolute values for protein fractions in sera from control mice (gms/100 ml).

	Time bled	Albumin	Globulin		
			alpha	beta	gamma
Hours	0	4.51	0.41	0.56	0.15
	9	4.55	0.30	0.49	0.13
	24	4.16	0.42	0.55	0.19
Days	4	4.50	0.39	0.59	0.20
	8	4.40	0.33	0.60	0.22
	12	4.64	0.38	0.62	0.24
	16	4.54	0.45	0.59	0.20
	20	4.39	0.52	0.47	0.28
	24	4.60	0.48	0.47	0.24
	28	4.86	0.39	0.58	0.17
	32	4.98	0.28	0.48	0.22
	36	5.10	0.57	0.63	0.27
	40	4.97	0.47	0.59	0.22
	44	4.91	0.51	0.59	0.27
	48	4.91	0.57	0.74	0.30
	52	4.90	0.54	0.60	0.30
	56	4.03	0.49	0.64	0.30
	60	4.62	0.72	0.68	0.27
	64	4.07	0.56	0.63	0.29
	68	4.18	0.54	0.66	0.28
	72	4.55	0.77	0.87	0.30
	76	4.41	0.83	0.92	0.38
	80	4.44	0.71	0.87	0.31
	84	4.37	0.78	0.79	0.34

Table II. Electrophoretic analysis of sera from control mice (relative concentrations in per cent).

	Time bled	Albumin	Globulin			A/G ratio	Total protein (gms/100 ml)
			alpha	beta	gamma		
Hours	0	80.2	7.2	9.9	2.7	4.05	5.63
	9	83.1	5.6	8.9	2.4	4.92	5.48
	24	78.2	7.8	10.4	3.5	3.59	5.33
Days	4	79.3	6.9	10.4	3.5	3.82	5.67
	8	79.2	5.9	10.9	3.9	3.81	5.55
	12	79.0	6.4	10.5	4.0	3.76	5.87
	16	78.6	7.7	10.3	3.4	3.44	5.76
	20	77.5	9.2	8.3	5.0	3.48	5.67
	24	79.6	8.3	8.2	4.0	3.90	5.78
	28	81.1	6.5	9.7	2.7	4.29	5.91
	32	83.7	4.7	8.0	3.7	5.13	5.96
	36	77.6	8.7	9.6	4.2	3.46	6.57
	40	79.7	7.5	9.3	3.5	3.93	6.24
	44	78.4	8.1	9.4	4.2	3.63	6.27
	48	75.5	8.7	11.4	4.5	3.08	6.51
	52	77.5	8.5	9.4	4.7	3.44	6.32
	56	73.9	9.0	11.8	5.4	2.83	5.45
	60	73.4	11.5	11.8	4.4	2.75	6.29
	64	73.3	10.2	11.5	5.1	2.75	5.54
	68	74.1	9.4	11.9	4.9	2.86	5.64
	72	70.1	11.8	13.3	4.1	2.34	6.48
	76	67.3	13.1	14.0	5.7	2.06	6.57
	80	70.3	11.2	13.7	4.9	2.37	6.32
	84	69.7	12.4	12.6	5.4	2.23	6.27

Table III. Electrophoretic analysis of sera from mice infected with the plerocercoids of Spirometra mansoni (relative concentrations in per cent).

Time, post infection		Albumin	Globulin			A/G ratio	Total protein (gms/100 ml)
			Alpha	beta	gamma		
Hours	3	67.4	11.1	17.2	5.3	1.74	6.30
	12	68.2	11.7	14.1	5.9	2.13	5.80
	24	65.9	11.4	17.0	5.7	2.00	6.00
Days	4	61.3	12.3	19.8	6.6	1.79	5.80
	8	58.8	12.6	19.3	9.2	1.51	5.52
	12	63.9	13.1	17.2	5.7	1.50	6.24
	16	66.4	11.2	14.9	7.5	1.62	5.40
	20	67.9	10.7	14.6	6.8	1.39	6.00
	24	59.8	10.3	19.5	10.3	1.56	5.55
	28	59.1	13.9	13.9	13.0	1.62	5.61
	32	62.0	12.0	14.0	12.0	1.24	5.61
	36	71.0	9.8	11.0	8.5	1.36	5.73
	40	68.5	8.1	15.3	8.1	1.52	7.32
	44	61.8	10.3	14.0	14.0	1.61	8.16
	48	62.9	10.7	15.0	11.4	1.66	7.95
	52	63.8	10.6	13.8	11.9	1.93	8.25
	56	58.1	10.8	17.7	13.3	1.00	5.82
	60	63.3	8.0	15.3	13.3	1.11	7.95
	64	61.5	10.3	16.2	11.9	1.18	7.50
	68	67.9	6.7	14.9	10.4	1.33	7.65
	72	58.7	10.3	15.9	15.1	0.97	8.25
	76	57.6	12.0	18.4	12.0	1.00	5.43
	80	54.4	10.7	20.4	14.6	1.13	5.55
	84	55.6	11.1	18.9	14.4	0.94	5.55

Table IV. Absolute values for protein fractions in sera from mice infected with plerocercoids of Spirometra mansoni (gms/100 ml).

Time post infection		Albumin	Globulin		
			alpha	beta	gamma
Hours	3	4.25	0.70	1.08	0.33
	12	3.96	0.68	0.82	0.34
	24	3.95	0.68	1.02	0.34
Days	4	3.56	0.71	1.15	0.38
	8	3.25	0.70	1.07	0.51
	12	3.99	0.82	1.07	0.36
	16	3.59	0.60	0.80	0.41
	20	4.07	0.64	0.88	0.41
	24	3.32	0.57	1.08	0.57
	28	3.31	0.78	0.78	0.73
	32	3.48	0.67	0.79	0.67
	36	4.07	0.56	0.63	0.49
	40	5.01	0.59	1.12	0.59
	44	5.04	0.84	1.14	1.14
	48	5.00	0.85	1.19	0.91
	52	5.26	0.87	1.14	0.98
	56	3.38	0.63	1.03	0.77
	60	5.03	0.64	1.22	1.06
	64	4.61	0.77	1.22	0.89
	68	5.19	0.51	1.14	0.80
	72	4.84	0.85	1.31	1.25
	76	3.13	0.65	1.00	0.65
	80	3.02	0.59	1.13	0.81
	84	3.09	0.62	1.05	0.80

Table V. Electrophoretic analysis of sera from mice infected with the plerocercoids of *Spirometra urichi* (relative concentrations in per cent).

Time, post infection		Albumin	Globulin			A/G ratio	Total protein (gms/100 ml)
			Alpha	beta	gamma		
Hours	3	63.5	9.4	19.8	7.3	1.74	6.60
	12	68.0	8.3	16.7	6.9	2.13	6.60
	24	66.6	10.7	15.5	7.1	2.00	5.61
Days	4	64.2	10.4	18.9	6.6	1.79	5.55
	8	60.2	16.5	15.5	7.8	1.51	7.05
	12	60.0	14.5	19.1	6.4	1.50	6.45
	16	61.8	13.2	19.7	5.3	1.62	5.82
	20	58.3	15.5	17.5	8.7	1.39	5.82
	24	60.9	13.0	16.0	10.1	1.56	5.61
	28	61.9	11.9	15.5	10.7	1.62	5.55
	32	55.4	12.2	18.9	13.5	1.24	5.49
	36	57.6	13.0	17.4	12.0	1.36	5.79
	40	60.3	11.1	16.7	11.9	1.52	8.19
	44	61.7	10.0	17.5	10.8	1.61	8.19
	48	62.4	14.0	14.0	9.6	1.66	8.19
	52	65.9	9.8	13.0	11.4	1.93	7.11
	56	50.0	17.2	19.4	13.4	1.00	6.06
	60	52.7	16.4	20.0	10.9	1.11	6.00
	64	54.1	15.6	17.4	12.8	1.18	6.21
	68	57.0	11.4	19.6	12.0	1.33	6.21
	72	49.2	15.8	21.0	14.1	0.97	6.48
	76	50.0	16.4	19.2	14.4	1.00	6.00
	80	53.0	12.7	21.5	12.7	1.13	6.21
	84	48.4	12.5	22.7	16.4	0.94	6.48

Table VI. Absolute values for protein fractions in sera from mice infected with plerocercoids of Spirometra urichi (gms/100 ml).

Time post infection		Albumin	Globulin		
			alpha	beta	gamma
Hours	3	4.19	0.62	1.31	0.48
	12	4.49	0.55	1.10	0.46
	24	3.74	0.60	0.87	0.40
Days	4	3.56	0.57	1.05	0.37
	8	4.24	1.16	1.09	0.55
	12	3.87	0.94	1.23	0.41
	16	3.60	0.77	1.14	0.31
	20	3.40	0.90	1.02	0.51
	24	3.42	0.73	0.90	0.57
	28	3.44	0.66	0.86	0.59
	32	3.04	0.67	1.04	0.74
	36	3.34	0.75	1.01	0.69
	40	4.94	0.91	1.37	0.97
	44	5.05	0.82	1.43	0.88
	48	5.11	1.15	1.15	0.79
	52	4.69	0.70	0.92	0.81
	56	3.03	1.04	1.18	0.81
	60	3.16	0.98	1.20	0.65
	64	3.36	0.97	1.08	0.79
	68	3.54	0.71	1.22	0.75
	72	3.19	1.02	1.36	0.91
	76	3.00	0.98	1.15	0.86
	80	3.29	0.79	1.34	0.79
	84	3.14	0.81	1.47	1.06

Table VII. Electrophoretic analysis of sera from mice infected with the plerocercoids of Spirometra mansonoides "A" (relative concentrations in per cent).

Time, post infection		Albumin	Globulin			A/G ratio	Total protein (gms/100 ml)
			alpha	beta	gamma		
Hours	3	61.5	13.8	16.9	7.7	1.59	5.91
	12	64.8	15.7	20.0	8.3	1.84	6.00
	24	58.8	15.1	16.0	10.0	1.43	6.15
Days	4	61.6	13.6	16.8	8.0	1.60	6.21
	8	50.1	19.2	19.9	10.8	1.00	6.00
	12	50.4	18.4	18.5	12.6	1.01	6.39
	16	48.8	19.8	11.2	13.2	0.95	6.30
	20	47.1	14.7	22.7	15.7	0.89	6.00
	24	55.8	13.0	19.6	11.6	1.26	5.82
	28	55.5	12.7	19.8	11.9	1.25	6.09
	32	55.0	14.7	17.1	13.2	1.22	5.82
	36	57.1	13.7	17.1	12.2	1.33	6.15
	40	48.7	14.6	20.3	16.5	0.95	6.30
	44	50.8	16.4	19.6	13.2	1.03	6.00
	48	47.7	14.7	23.9	13.8	0.91	6.09
	52	49.6	12.4	21.2	16.8	0.98	6.39
	56	43.9	17.5	18.4	20.2	0.78	6.48
	60	47.8	17.7	18.6	15.9	0.92	5.82
	64	51.8	17.7	17.7	12.8	1.07	5.49
	68	45.8	15.3	22.2	16.7	0.85	5.91
	72	45.8	18.7	18.7	16.9	0.85	5.91
	76	43.7	17.1	22.2	17.1	0.78	5.76
	80	50.0	19.6	17.1	13.3	1.00	6.21
	84	51.0	14.1	20.8	14.1	1.04	5.82

Table VIII. Absolute values for protein fractions in sera from mice infected with plerocercoids of Spirometra mansonoides "A" (gms/100 ml).

Time post infection		Albumin	Globulin		
			alpha	beta	gamma
Hours	3	3.63	0.82	1.00	0.46
	12	3.18	0.94	1.20	0.68
	24	3.62	0.93	0.98	0.62
Days	4	3.83	0.84	1.04	0.50
	8	3.01	1.15	1.19	0.65
	12	3.22	1.18	1.18	0.81
	16	3.07	1.25	1.15	0.83
	20	2.83	0.88	1.35	0.94
	24	3.25	0.76	1.14	0.65
	28	3.38	0.77	1.21	0.73
	32	3.20	0.86	1.00	0.77
	36	3.51	0.84	1.05	0.75
	40	3.07	0.92	1.28	1.04
	44	3.05	0.98	1.18	0.79
	48	2.90	0.90	1.46	0.84
	52	3.17	0.79	1.35	1.07
	56	2.85	1.13	1.19	1.31
	60	2.78	1.03	1.08	0.93
	64	2.84	0.97	0.97	0.70
	68	2.71	0.90	1.31	0.99
	72	2.71	1.11	1.11	1.00
	76	2.52	0.98	1.28	0.98
	80	3.11	1.22	1.06	0.83
	84	2.97	0.82	1.21	0.82

Table IX. Electrophoretic analysis of sera from mice infected with the plerocercoids of Spirometra mansonoides "B" (relative concentrations in per cent).

Time, post infection		Albumin	Globulin			A/G ratio	Total protein (gms/100 ml)
			alpha	beta	gamma		
Hours	3	62.9	10.5	16.1	10.5	1.70	6.09
	12	66.7	12.5	12.5	8.3	2.00	5.55
	24	66.7	11.6	15.9	5.8	2.00	4.80
Days	4	55.3	16.5	18.4	9.7	1.24	5.70
	8	47.7	17.4	24.4	10.5	0.91	5.25
	12	61.7	10.6	22.3	5.3	1.61	5.91
	16	61.8	12.7	19.1	6.4	1.62	5.52
	20	62.6	12.1	18.2	7.1	1.67	5.40
	24	61.1	9.7	21.0	8.3	1.57	5.34
	28	55.6	11.1	18.5	14.8	1.25	5.34
	32	65.1	12.3	14.8	7.4	1.87	5.49
	36	54.2	14.6	18.8	12.5	1.18	5.79
	40	62.8	10.5	18.6	8.1	1.69	4.80
	44	56.7	10.0	18.9	14.4	1.31	5.61
	48	53.3	15.2	20.0	11.4	1.14	5.16
	52	50.6	14.9	23.0	11.5	1.02	5.61
	56	43.4	15.1	22.6	18.9	0.77	5.61
	60	44.3	18.3	25.2	12.2	0.80	5.79
	64	47.2	14.9	22.4	15.5	0.89	5.82
	68	54.8	16.4	17.8	11.0	1.21	6.00
	72	54.3	15.0	15.7	15.0	1.19	5.82
	76	60.0	11.7	16.6	11.7	1.50	6.00
	80	57.9	14.7	17.8	9.6	1.38	6.00
	84	49.3	18.3	16.2	16.2	0.97	6.21

Table X. Absolute values for protein fractions in sera from mice infected with plerocercoids of Spirometra mansonoides "B" (gms/100 ml).

Time post infection		Albumin	Globulin		
			alpha	beta	gamma
Hours	3	3.83	0.64	0.98	0.64
	12	3.70	0.69	0.69	0.46
	24	3.20	0.56	0.76	0.28
Days	4	3.15	0.94	1.05	0.55
	8	2.50	0.91	1.28	0.55
	12	3.65	0.63	1.32	0.31
	16	3.41	0.70	1.05	0.35
	20	3.38	0.65	0.98	0.38
	24	3.26	0.52	1.12	0.44
	28	2.97	0.59	0.99	0.79
	32	3.57	0.68	0.81	0.41
	36	3.14	0.85	1.09	0.72
	40	3.01	0.50	0.89	0.39
	44	3.18	0.56	1.06	0.81
	48	2.75	0.78	1.03	0.59
	52	2.84	0.84	1.29	0.65
	56	2.43	0.85	1.27	1.06
	60	2.56	1.06	1.46	0.71
	64	2.75	0.87	1.30	0.90
	68	3.29	0.98	1.07	0.66
	72	3.16	0.87	0.91	0.87
	76	3.60	0.70	1.00	0.70
	80	3.47	0.88	1.07	0.58
	84	3.06	1.14	1.01	1.01

Table XI. Absolute values for protein fractions in sera from sham injected rabbits (gms/100 ml).

Weeks post immunization	Total protein	Albumin	Globulin			A/G ratio
			alpha	beta	gamma	
0	6.94	4.67	0.84	0.79	0.64	2.06
4	7.16	4.88	0.88	0.72	0.69	2.13
6	7.14	4.91	0.71	0.80	0.72	2.20

Table XII. Electrophoretic analysis of sera from sham injected rabbits (concentrations in per cent).

Weeks post immunization	Albumin	Globulin		
		alpha	beta	gamma
0	67.3	12.1	11.4	9.2
4	68.1	12.3	10.0	9.6
6	68.7	10.0	11.2	10.1

Table XIII. Absolute values for protein fractions in sera from rabbits immunized against Spirometra mansoni (gms/100 ml).

Weeks post immunization	Total protein	Albumin	Globulin			A/G ratio
			alpha	beta	gamma	
0	7.25	4.72	0.83	0.89	0.89	1.87
4	7.17	4.41	1.01	0.72	1.03	1.60
6	7.05	4.39	0.80	0.66	1.21	1.65

Table XIV. Electrophoretic analysis of sera from rabbits immunized against Spirometra mansoni (concentration in per cent).

Weeks post immunization	Albumin	Globulin		
		alpha	beta	gamma
0	65.1	11.4	11.1	12.3
4	61.5	14.1	10.0	14.4
6	62.2	11.4	9.3	17.1

Table XV. Absolute values for protein fractions in sera from rabbits immunized against *Spirometra urichi* (gms/100 ml).

Weeks post immunization	Total protein	Albumin	Globulin			A/G ratio
			alpha	beta	gamma	
0	6.99	5.04	0.65	0.61	0.69	2.58
4	7.25	4.70	0.83	0.60	1.12	1.84
6	7.38	4.27	0.63	0.76	1.72	1.37

Table XVI. Electrophoretic analysis of sera from rabbits immunized against Spirometra urichi (concentrations in per cent).

Weeks post immunization	Albumin	Globulin		
		alpha	beta	gamma
0	72.1	9.3	8.7	9.8
4	64.8	11.4	8.3	15.5
6	57.8	8.6	10.3	23.3

Table XVII. Absolute values for protein fractions in sera from rabbits immunized against Spirometra mansonoides "A" (gms/100 ml).

Weeks post immunization	Total protein	Albumin	Globulin			A/G ratio
			alpha	beta	gamma	
0	6.75	4.60	0.80	0.75	0.64	2.10
4	6.60	4.30	0.79	0.85	0.67	1.87
6	6.90	4.44	0.77	0.85	0.83	1.81

Table XVIII. Electrophoretic analysis of sera from rabbits immunized against Spirometra mansonoides "A" (concentrations in per cent).

Weeks post immunization	Albumin	Globulin		
		alpha	beta	gamma
0	67.7	11.8	11.1	9.4
4	65.1	11.9	12.9	10.1
6	64.4	11.2	12.3	12.1

Table XIX. Absolute values for protein fractions in sera from rabbits immunized against Spirometra mansonoides "B" (gms/100 ml).

Weeks post immunization	Total protein	Albumin	Globulin			A/G ratio
			alpha	beta	gamma	
0	7.25	4.92	0.70	0.38	1.25	2.12
4	6.99	4.65	0.66	0.40	1.29	1.99
6	7.38	4.72	0.72	0.52	1.42	1.73

Table XX. Electrophoretic analysis of sera from rabbits immunized against Spirometra mansonoides "B" (concentrations in per cent).

Weeks post immunization	Albumin	Globulin		
		alpha	beta	gamma
0	67.9	9.6	5.2	17.3
4	66.5	9.4	5.7	18.4
6	63.4	9.8	7.0	19.3

Figure 1. Pattern of changes in serum proteins in control mice.

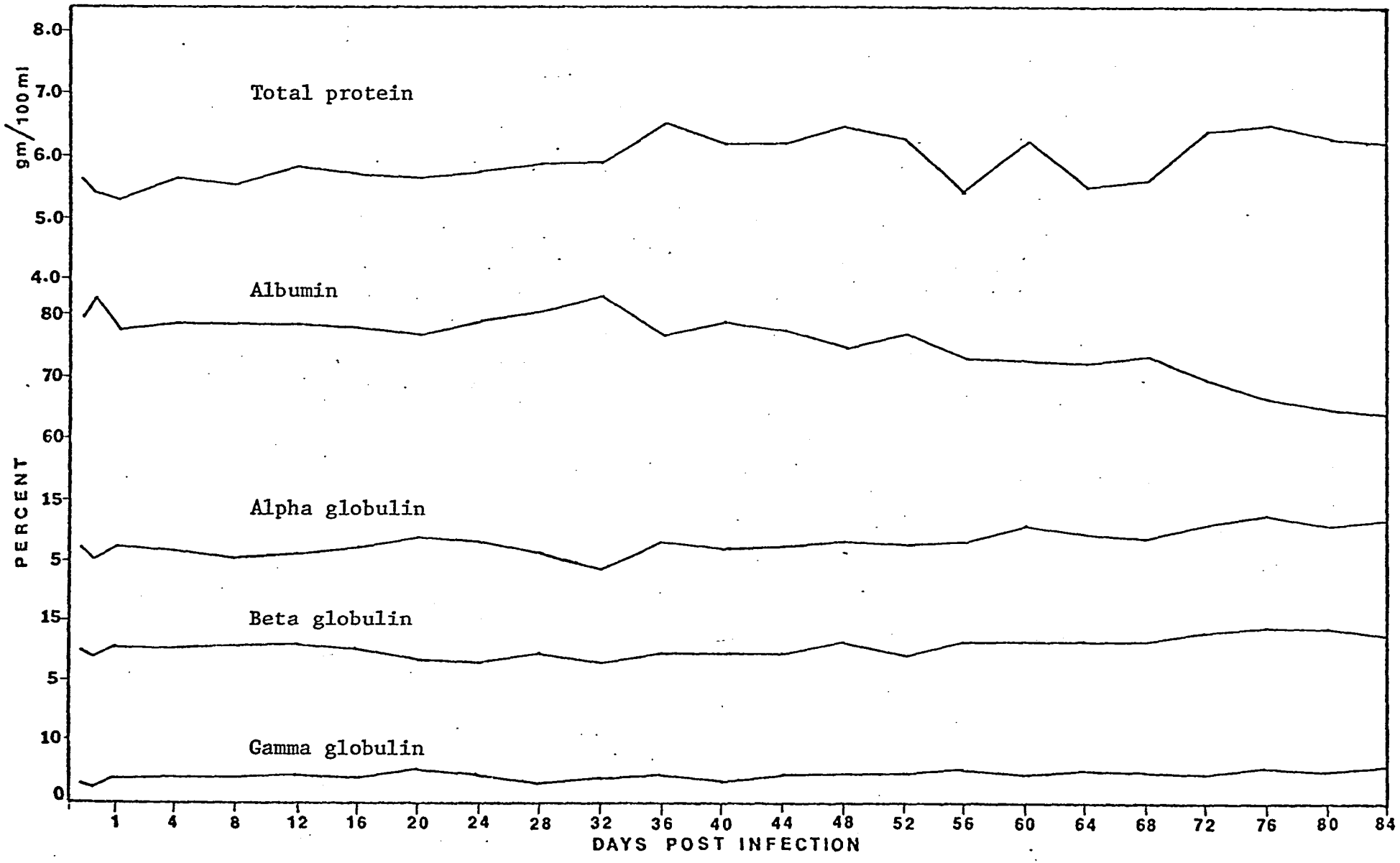


Figure 2. Pattern of changes in serum proteins in mice experimentally infected with the plerocercoids of Spirometra mansoni.

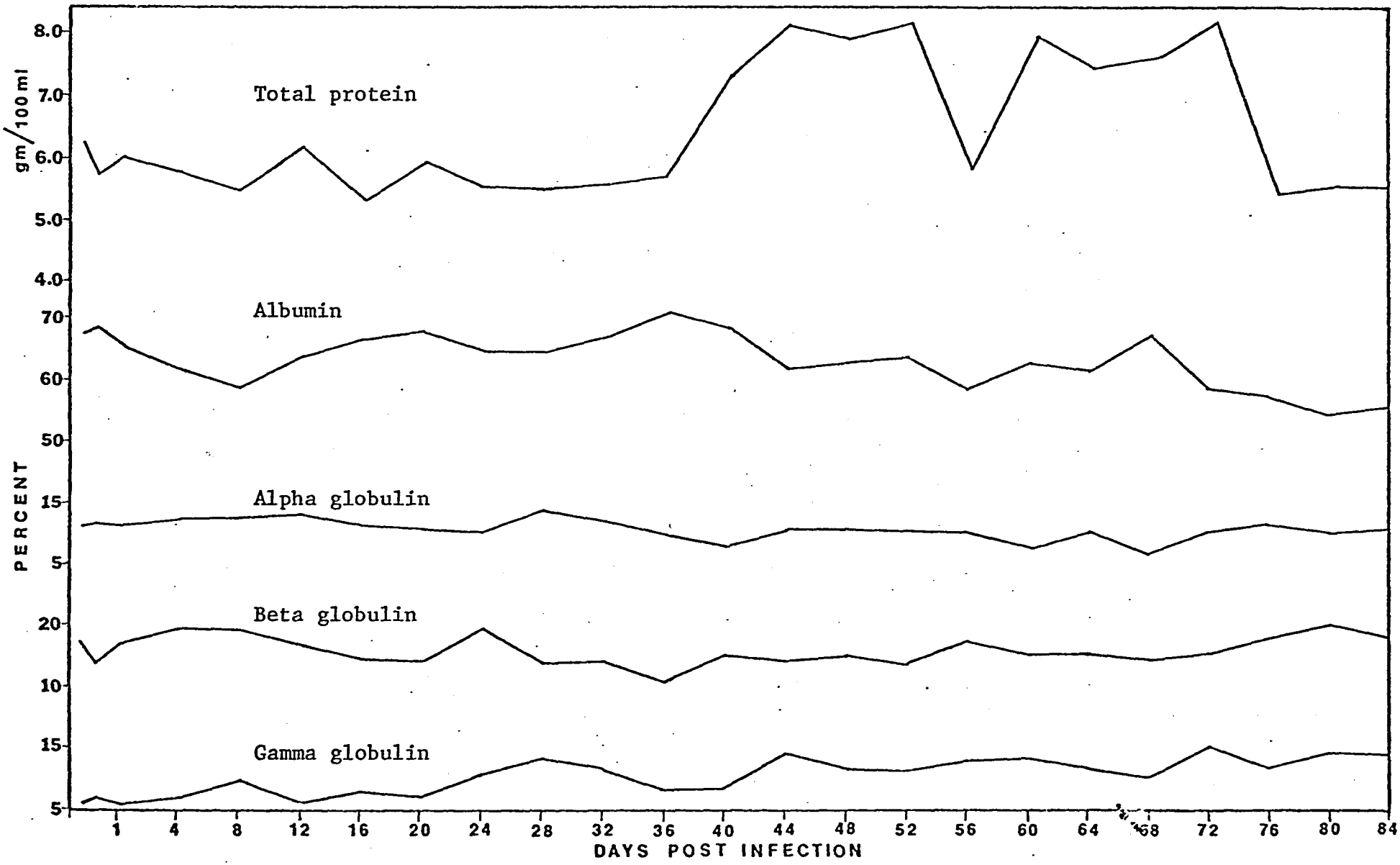


Figure 3. Pattern of changes in serum proteins in mice experimentally infected with the plerocercoids of Spirometra urichi.

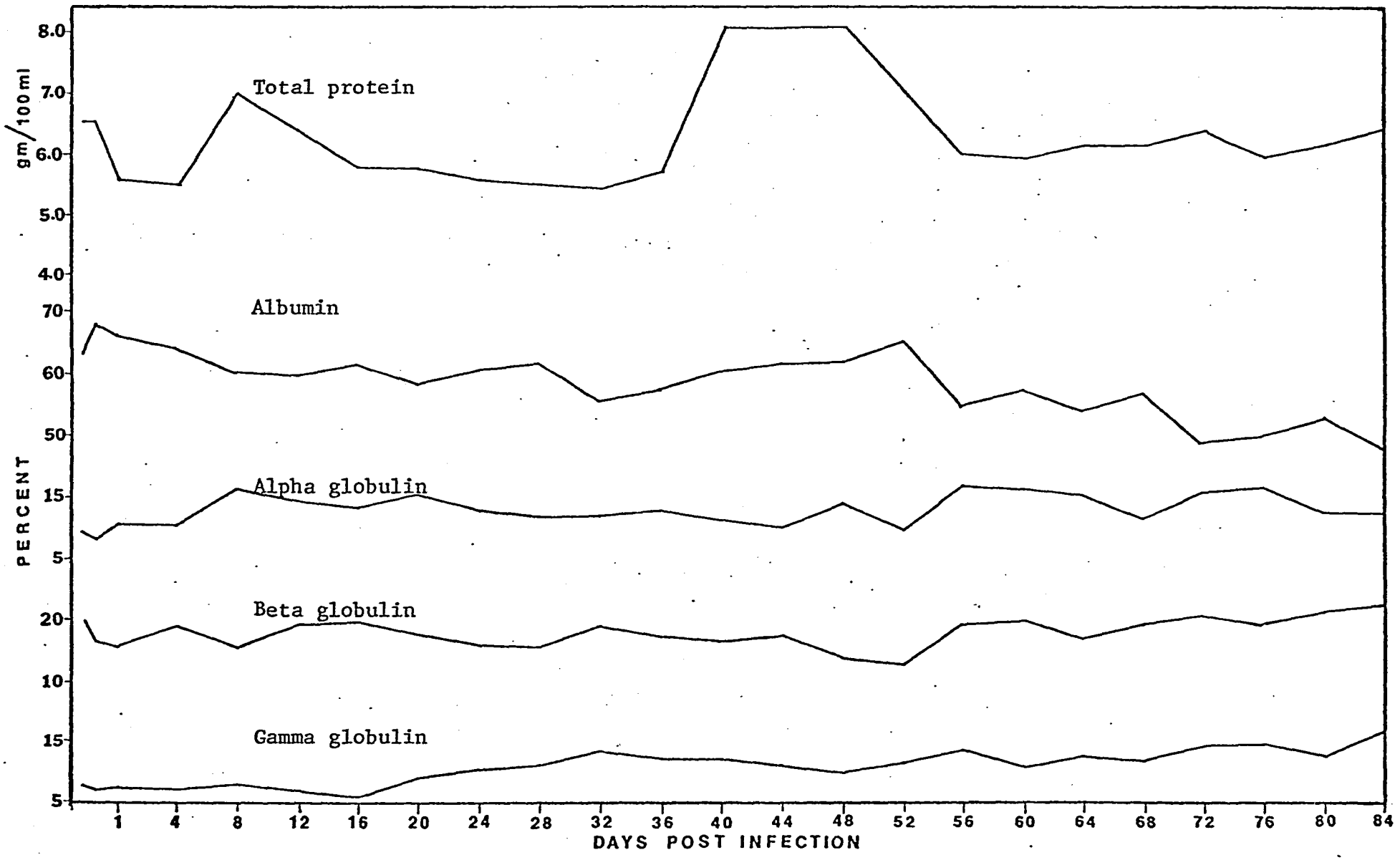


Figure 4. Pattern of changes in serum proteins in mice experimentally infected with the plerocercoids of Spirometra mansonoides "A".

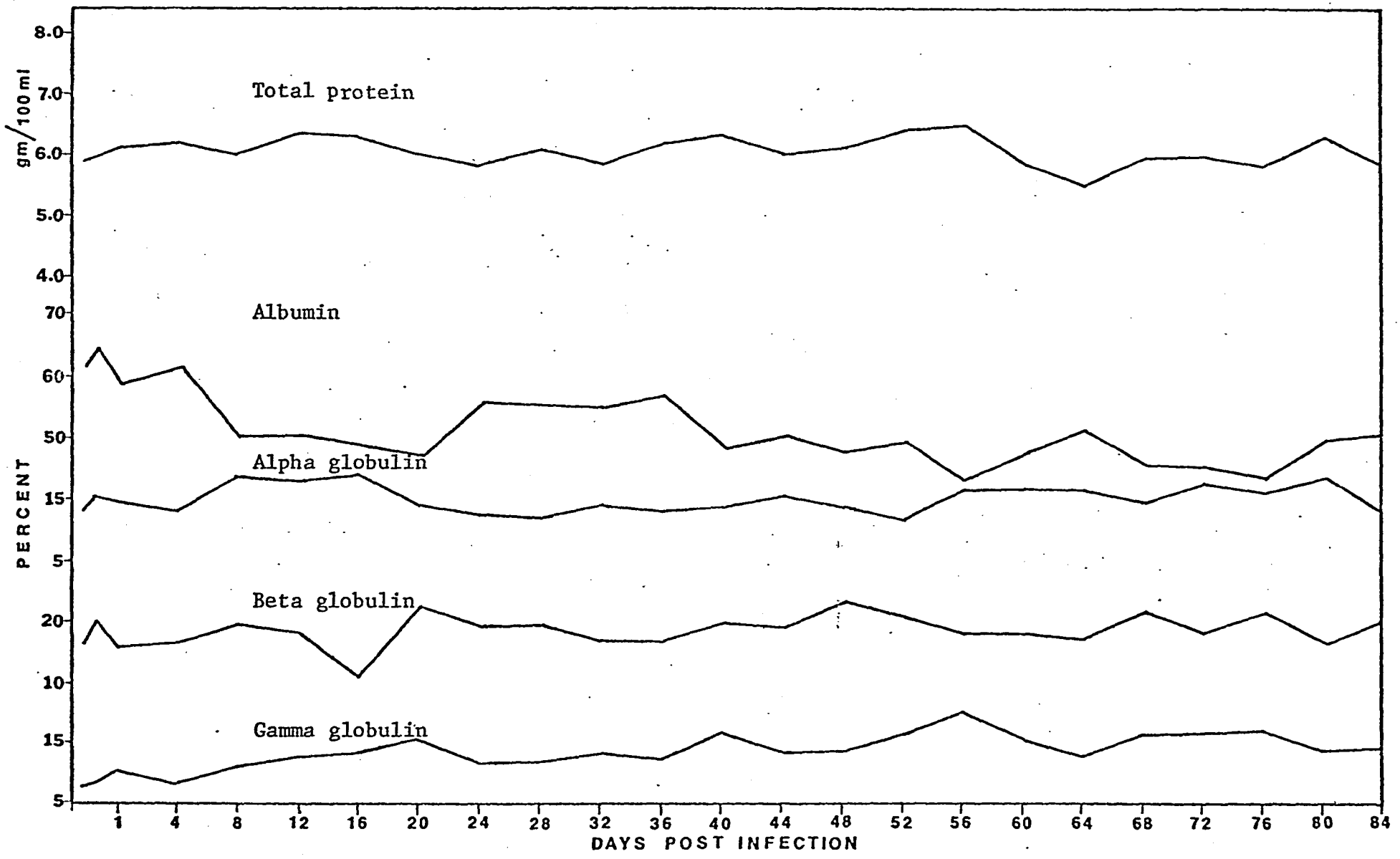
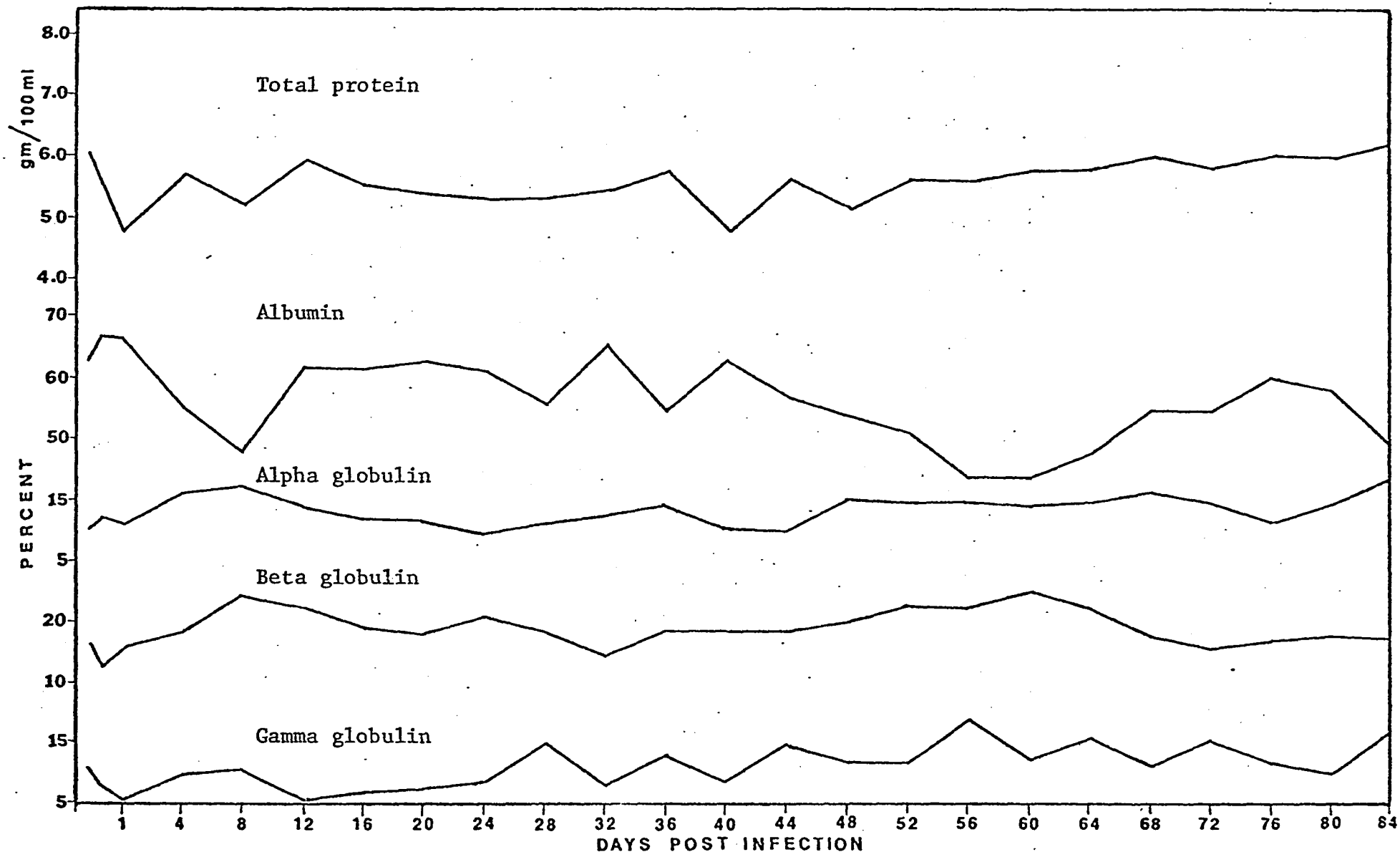


Figure 5. Pattern of changes in serum proteins in mice experimentally infected with the plerocercoids of Spirometra mansonoides "B".



EXPLANATION OF PLATES

Representation of precipitin bands which formed when immune sera from mice experimentally infected with plerocercoids and rabbits passively immunized against the plerocercoids were matched with their homologous antigens. IM=immune sera; AN-antigen; SA=saline.

Plate I. Double gel diffusion of sera from mice experimentally infected with the plerocercoids of Spirometra

Fig. a. Pattern of precipitin bands which formed with the sera from mice infected with Spirometra mansoni at 44 days

Fig. b. Pattern of precipitin bands which formed with the sera from mice infected with Spirometra urichi at 40 days

Fig. c. Pattern of precipitin bands which formed with the sera from mice infected with Spirometra mansonoides "A" at 40 days

Fig. d. Pattern of precipitin bands which formed with the sera from mice infected with Spirometra mansonoides "B" at 40 days

Plate II. Double gel diffusion of sera from rabbits passively immunized against the plerocercoids of Spirometra

Fig. a. Precipitin bands which formed with the sera from rabbits immunized against Spirometra mansoni six weeks post immunization

Fig. b. Pattern of precipitin bands which formed with the sera from rabbits immunized against Spirometra urichi six weeks post immunization

Fig. c. Pattern of precipitin bands which formed with the sera from rabbits immunized against Spirometra mansonoides "A"

Fig. d. Pattern of precipitin bands which formed with the sera from rabbits immunized against Spirometra mansonoides "B"

Plate I

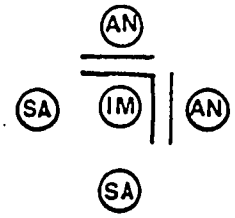


Fig. a

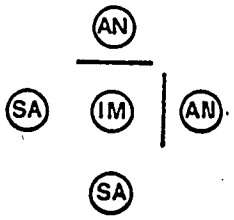


Fig. b

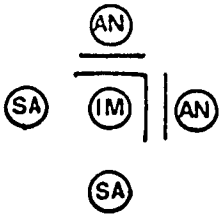


Fig. c

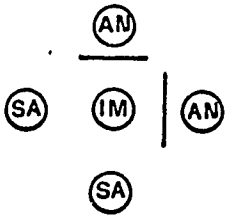


Fig. d

Plate II

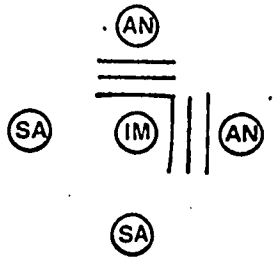


Fig. a

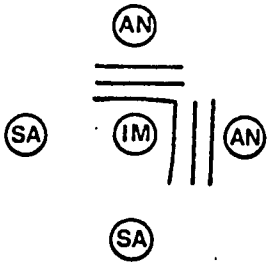


Fig. b

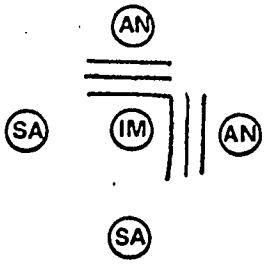


Fig. c

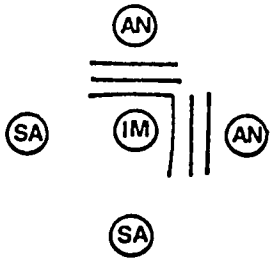


Fig. d

DISCUSSION AND CONCLUSIONS

A study by Sadum, et al. (1965) showed that sera from mice injected subcutaneously with the plerocercoids of S. mansonoides exhibited significantly lower total protein values than controls at intervals of 11, 16 and 18 weeks after infection. Their electrophoretic analyses of these sera indicated that neither albumin nor the globulins were responsible for this diminution. They further noted that gamma globulins, which include most of the circulating antibodies, were not increased in the infected animals. The results of the present study demonstrate, however, that sera from animals infected with and immunized against the plerocercoids of three species of Spirometra, including S. mansonoides, do in fact have altered serum protein concentrations and form precipitates in agar gel.

Factors which may attribute to the differences in results of these two studies are the interval during the experimental period when serum samples were taken and the route of infection. It has been previously noted in the results of this study that total protein concentrations are not maintained at a constant level and that these values are at times below those of the controls. Therefore, it is possible that protein concentrations determined by Sadun and his co-workers at 11, 16 and 18 weeks were at times when these levels were depressed.

Secondly, in the present study, mice were fed the scolices of plerocercoids. The difference in the route of imposing infections may account for the results that demonstrate changes in the protein

concentrations and the presence of precipitating antibodies during the course of the infection.

When plerocercoids are fed, they are capable of penetrating the gut in as little as 15 minutes. As they pass through the gut, the migration is in a stepped tangential plane. This oblique path between the interface of cell layers prevents the intestinal contents from spilling into the peritoneal cavity. Once in the peritoneal cavity, the plerocercoids move freely, eventually penetrate the parietal peritoneum and migrate into the musculature and fascial layer of the skin. Plerocercoids were noted to be present in the skin fascia of mice in as little as one hour after feeding.

The initial passage of the larvae through the gut prior to establishment at the site of predilection is concluded to result in a greater exercitation of the immune mechanisms of the host than would result from subcutaneous injections of plerocercoids. During this rapid migration it must be assumed that large quantities of secretory materials are being released by the worms. Passage of these larvae through the peritoneal cavity presumably would bring these antigenic substances more quickly into the circulation and in contact with tissues that produce antibodies.

Electrophoretic Studies

Infections with the plerocercoids of the three species of Spirometra were reflected by changes in the serum protein fractions. The over-all albumin concentrations in sera from mice infected were below the values for the controls as determined by electrophoresis. Conversely, all globulin fractions in sera from infected mice

exhibited concentrations that were above those of the controls. These differences are considered to be a reflection of the physiological state in the infected animals induced by the plerocercoids of these species.

It has been previously noted that major changes occur in one or more of the components of serum proteins following infection with various helminths. The pattern of these changes varies with the host and parasite. Luetscher (1947) noted that electrophoretic patterns are indicative of the host's reaction to infection or injury and not characteristic of the specific disease. He further indicated that in almost every pathologic state there is a relative or absolute decrease in serum albumin. The changes in serum proteins of sera from infected animals in this study reflect relative decreases in the serum albumin and relative increases in one or more of the globulins.

Albumin concentrations in sera from mice infected with the plerocercoids of Spirometra were not greatly reduced. The final values for albumin were approximately ten per cent below the initial concentrations. Comparable increases in the globulin fractions were recorded.

Various studies have indicated that the liver is the site of albumin synthesis (Madden and Wipple, 1940; Peters and Anfinsen, 1950). Therefore, damage to the liver parenchyma by parasitic invasion would greatly affect the synthesis of albumin.

Spirometrid plerocercoids seldom invade the liver, therefore, the depressed albumin concentrations are not likely attributable to liver damage. The possibility exists that toxic by-products of the larvae cause some cellular or functional change that would be

reflected by decreased albumin anabolism.

Elevated concentrations of the alpha globulins is another manifestation of spirometrid infections. All experimental groups had alpha levels above those exhibited by the controls.

The results of studies by Shedlovsky and Scudder (1942) indicate that increased alpha globulins occur when there is considerable inflammation. Examination of loci where spirometrid plerocercoids have been removed from infected mice occasionally show signs of inflammation or tissue damage. These inflammatory reactions are more noticable on the whitish dermal layer of the skin than in the musculature where the redness would be less evident. These regions are not extensive, but are generally restricted to the area in immediate contact with the larvae. In an individual locus, the amount of inflammation would be regarded as minor, however, when considered collectively, reactions in several loci may be sufficient to raise the total response to a substantial level.

Precipitate formation on larvae *in situ* enhance the inflammatory response. Taliaferro and Sarles (1939) observed that precipitates form in and around the larvae of *N. muris in situ*. In addition to immobilizing and sometimes killing these larvae, the precipitates increased the intensity of the inflammatory response.

Precipitates also form on spirometrid plerocercoids *in situ* as noted by Mueller (1961). During the present study and in life cycle studies with various species of *Spirometra*, similar precipitates have been observed on these larvae.

When spirometrid larvae are transferred to new hosts without being washed several times in distilled water or saline,

precipitates are apparently still adherent to the worms. In order to circumvent the inflammatory reactions due to the transferral of precipitates from one host to another, larval scolices which were administered per os to the animals of this study were washed several times in saline. Any remaining precipitate would probably have been eliminated as the larvae passed through the wall of the digestive system.

Electrophoretic and immunoelectrophoretic studies have demonstrated that reaginic antibodies are identified with the beta globulins (Hereman and Vaerman, 1962; Fireman, et al., 1963). These antibodies include those which sensitize cells of the skin and connective tissue.

Reaginic antibodies have been demonstrated in helminthic infections involving N. brasiliensis (Ogilvie, 1964, 1967; Jones and Ogilvie, 1967; Wilson, 1967), T. spiralis (Briggs, 1963; Sadun, et al., 1968), S. ratti (Goldgraber and Lewert, 1965) and by passive immunization with antigens from T. spiralis (Briggs, 1963) and S. ratti (Goldgraber and Lewert, 1965).

Increases in the beta globulins were observed in mouse sera from all the infected groups in the present study. Two major beta peaks occurred in the S. mansonoides "A" and "B" groups. These peaks were evident at about one and seven to eight weeks post infection. The Spirometra mansoni group exhibited beta peaks at four, 24 and 80 days post infection. Although sera from the S. urichi group exhibited no peak beta concentration during the study period, final values were elevated above the initial level.

No specific tests were performed in this study to detect or

determine quantities of reaginic antibodies, but results of other experiments (Corkum and Henson, unpublished) have indicated their presence in mice infected with the plerocercoids of S. urichi.

Ogilvie (1967) reported the detection of reaginic antibodies in rats shortly after immunity is acquired against larval N. brasiliensis infections. When secondary or repeated injections of larvae of this species were administered, anamnestic rises of these antibodies occurred.

If the peaks of beta globulin are indeed the result of reaginic antibody production, it would have to be assumed that during the intervals between the peaks, antibodies were removed from the circulation by absorption into the tissues or the production of antigenic stimulation was much reduced. Reaginic antibodies have been reported detectable for period up to seven months in persistent nematode infections (Ogilvie, 1967). At this time it is speculative whether reagin production is the cause of elevated beta globulin concentrations in spirometrid infections, however, it is likely.

Gamma globulin increases were noted in the sera from infected mice. Patterns of gamma globulin increases were noted to be similar in the sera from infected mice in the Spirometra mansoni, S. urichi and S. mansonoides "B" groups. Twenty-four hours after oral infections, increased concentrations were evident in the gamma fraction. Following this initial rise, lasting for about four days, levels declined to a point below the initial concentrations. The depressed levels were maintained until day 16 at which time near maximum increases were attained 32 days post infection. A similar pattern was observed with the sera from the S. mansonoides "A" group,

however, the initial rise was protracted until day 20, followed by a decline and a secondary elevation that coincided with the rises noted in the other groups.

Gamma globulins contain most if not all of the precipitating antibodies. It is assumed that such antibodies were present in these sera prior to their appearance as precipitins because of the initial rises as suggested by the electrophoretic patterns. Apparently, they were in such low quantities during the early phase of the infection that they were not detectable as precipitins in gel agar. Also the fact that precipitates form about the plerocercoids in situ would indicate that some or most of these antibodies are removed from the circulation by adsorption onto the surface of the larvae. This in part would account for the decline of the gamma globulins after an initial rise. It was not unitl a secondary rise of approximately seven per cent above the initial concentrations of the gamma globulins were these antibodies available in sufficient quantities to form visible precipitins in gels.

Gel Diffusion

The detection of precipitating antibodies in these sera corroborates the findings of Mueller (1961), who noted that precipitates were formed on plerocercoids incubated in immune serum from mice chronically infected with the larvae of S. mansonoides. He further noted that plerocercoids migrate for about one month after introduction into mice, after which time the larvae come to lie in discrete bundles in the sites of predilection. The nature of this action by the larvae is not known, however, it is possible that

antibody production is the cause behind the cessation of movement in the host.

It is interesting to note that precipitates were detected almost on the same day in post infection sera from mice infected with the plerocercoids of all three species. This time interval would coincide with the cessation of larval movement in the hosts. Precipitating antibodies were detected at 40 days in the sera of mice infected with S. urichi and S. mansonoides "A" and "B" and at 44 days in the sera from mice infected with Spirometra mansoni.

Two precipitin bands were observed with the sera from the Spirometra mansoni and S. mansonoides "A" groups and one band with the sera from the S. urichi and S. mansonoides "B" groups.

Several factors may attribute to the differences in the number of bands demonstrable in the sera from the different groups. Morphologically, the plerocercoids of these species are indistinguishable, however, consistent differences in their size have been observed. Larvae of the different species will exhibit size variation after the same period of development in the laboratory host. For example, the plerocercoids of Spirometra mansoni are very robust and attain lengths of 30 centimeters after a month or more in laboratory mice thus demonstrating a growth rate that exceeds the other species cultured in a like manner.

The plerocercoids of S. mansonoides on the other hand are smaller, 20 centimeters or more in length, whereas, those of S. urichi are approximately half the size, ten to 15 centimeters in length, of the other species. Characteristically the scolices of the latter species are smaller and the body thread-like, while the others

have heavy scolices and the bodies are ribbon-like.

The size and growth rate of the plerocercoids of Spirometra mansoni and S. mansonoides "A" and "B" may provide more antigens in the form of excretory-secretory materials for antibody production. The diminutive size of S. urichi would release lesser amounts of the same antigens into the host. Antibodies subsequently produced against these antigens would be in such low quantities that they are not detectable as precipitins in gels or at most result in only one precipitin band being formed.

The sera from the S. mansonoides "A" group formed two precipitin bands in gels, whereas, the "B" group formed only one. The appearance of only one band with the sera of this group may have been influenced by the death of four mice prior to and during the time antibodies were detected. All members in a group would not be expected to respond identically to antigenic stimuli, thus the mice that died were possibly among those that had greater immunological capabilities to antigenic stimulation. This would have thereby reduced the quantities of antibodies in the pooled sera that would have reacted when matched with the homologous antigen. No deaths were recorded for the Spirometra mansoni group and only one death in each of the S. urichi and S. mansonoides "A" groups.

Passive Immunization

Passive immunization of rabbits was a further attempt to characterize the antigenicity of plerocercoids of the three species. Immunization of the rabbits with lyophilized whole larval antigen resulted in no increase in the total serum protein concentrations. Changes in the protein fractions were consistent in the sera of rabbits immunized against Spirometra mansoni and S. mansonoides "A" and "B". These changes were characterized by slight decreases, 3.0 per cent, in the albumin concentrations and a corresponding increase, 3.2 per cent, in the gamma globulins.

Albumin and gamma globulin concentrations in sera from the rabbit immunized against S. urichi exhibited much greater changes. A decrease of 14.3 per cent in the albumin levels and a 13.5 per cent increase in the gamma globulins was noted.

The results of gel diffusion studies with the sera from this rabbit indicate that most of the albumin and gamma globulin changes were not attributable to immunization with plerocercoid antigen and were nonspecific. If these changes had been a reflection of the immunization, a greater number of precipitin bands would have been formed. Instead, no increase in the number or intensity of precipitin bands was evident, for three precipitin bands were formed by each of the serum samples taken at the six week post immunization.

Intradermal Tests

Although the results of the skin tests were inconclusive, the reactions observed in some of the passively immunized mice indicate that delayed hypersensitivity exists. Delayed hypersensitivity reactions are characterized by induration, edema and necrosis. Reactions of this type were noted in sensitized mice, however, the intensity varied considerably between individuals tested. In some no reaction was observed.

It has been previously noted that the results of skin tests for other helminthic infections were highly variable. The difficulty of interpretation of the reactions is the principal shortcoming of these tests. Since there is a lack of standards, one must attempt to be objective when assigning values which denote a true response.

SUMMARY

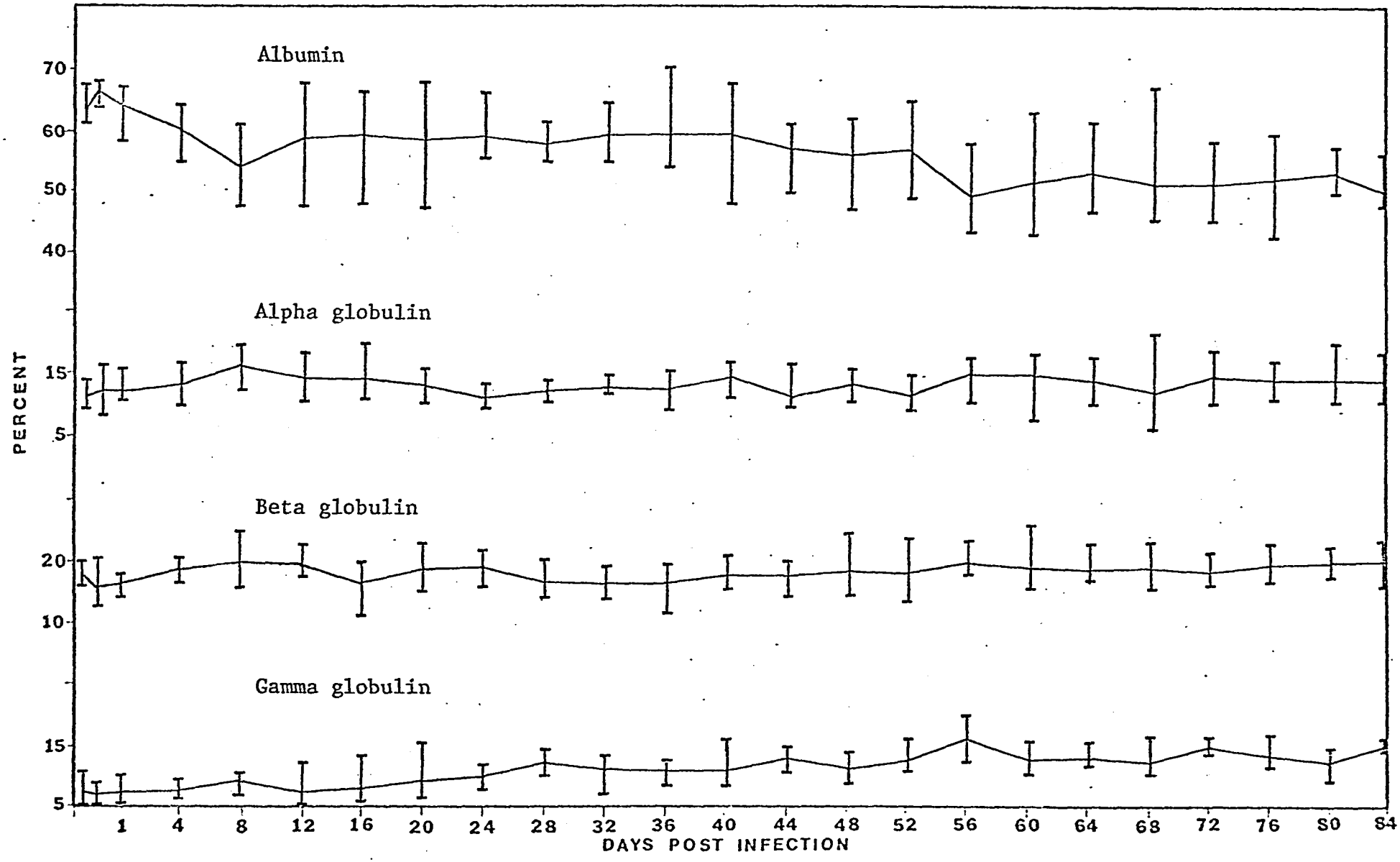
Summary Figure 6 shows the average of changes in serum protein fractions in the four experimentally infected groups. These changes were reflected by a gradual decline in the serum albumin, slightly increased values for the alpha and beta globulins and a gradual but continuous increase in the gamma globulins.

As previously noted, the clear delineation of the species of Spirometra on the basis of the usual morphological criteria alone is very difficult and has not been completely satisfactory. Also the results of life cycle studies have provided little additional help in separating the species. The almost complete lack of host specificity, coupled with the fact that the species from widely separated geographical localities can be reared experimentally in different allopatric hosts lends credence to the idea we are dealing with a single, widely distributed species.

One aspect of this study was to determine if immunological tests could be an aid in separating the various species of the genus Spirometra. The results of this study indicate, however, they are inseparable on the basis of the immunological tests.

While the host-plerocercoid relationship does not result in dramatic responses as previously noted in some helminthic infections, it is none the less perceptible. Larval infections in experimental animals induce subtle, but detectable, immunological responses.

Figure 6. Average of changes in serum protein fractions in mice experimentally infected with the plerocercoids of Spirometra mansoni, S. urichi and S. mansonoides "A" and "B".



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EXAMINATION AND THESIS REPORT

Candidate: Harry L. Henson, Jr.

Major Field: Invertebrate Zoology

Title of Thesis: Immunological Studies on the Genus Spirometra (Cestoda:Pseudophyllidea)

Approved:

Kenneth C Corkum
Major Professor and Chairman

R D Anderson
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Date of Examination:

26 June 1969